ARSENIC SPECIATION BY ION-EXCHANGE CHROMATOGRAPHY WITH ON-LINE DETERMINATION BY HYDRIDE GENERATION: ELECTRONIC MODIFICATIONS OF THE D.C. PLASMA BY PHOTON COUNTING

By

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ABSTRACT

A method using L-cysteine for the determination of arsenous acid (As(III)), arsenic acid (As(V)), monomethylarsonic acid (MMAA), and dimethylarsinic acid (DMAA) by hydride generation was demonstrated. The instrument used was a d.c. plasma atomic emission spectrometer (DCP-AES). Complete recovery was reported for As(III), As(V), and DMAA while 86% recovery was reported for MMAA. Detection limits were determined, as arsenic for the species listed previously, to be 1.2, 0.8, 1.1, and 1.0 $ng \bullet mL^{-1}$, respectively. Precision values, at 50 $ng \bullet mL^{-1}$ arsenic concentration, were 1.8%, 2.5%, 2.6% and 2.6% relative standard deviation, respectively.

The L-cysteine reagent was compared directly with the conventional hydride generation technique which uses a potassium iodide-hydrochloric acid medium. Recoveries using L-cysteine when compared with the conventional method provided the following results: similar recoveries were obtained for As(III), slightly better recoveries were obtained for As(V) and MMAA, and significantly better recoveries for DMAA. In addition, tall and sharp peak shapes were observed for all four species when using L-cysteine.

The arsenic speciation method involved separation by ion exchange - high performance liquid chromatography (HPLC) with on-line hydride generation using the L-cysteine reagent and measurement by DCP-AES. Total analysis time per sample was 12 min while the time between the start of subsequent runs was approximately 20 min. A binary gradient elution program, which incorporated the following two eluents: 0.01 and 0.5 mM tri-

sodium citrate both containing 5% methanol (v/v) and both at a pH of approximately 9, was used during the separation by HPLC. Recoveries of the four species which were measured as peak area, and were normalized against As(III), were 88%, 29%, and 40% for DMAA, MMAA and As(V), respectively. Resolution factors between adjacent analyte peaks of As(III) and DMAA was 1.1; DMAA and MMAA was 1.3; and MMAA and As(V) was 8.6.

During the arsenic speciation study, signals from the d.c. plasma optical system were measured using a new photon-signal integrating device. The new photon integrator developed and built in this laboratory was based on a previously published design which was further modified to reflect current available hardware. This photon integrator was interfaced to a personal computer through an A/D convertor. The photon integrator has adjustable threshold settings and an adjustable post-gain device.

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INTRODUCTION

Chemistry is a very old science in which analytical chemistry involving trace analysis is a very young branch. Analytical chemistry as it name suggests deals with the analysis of chemicals in an applied sense. It is not based so much on promoting and developing theories but on the application of theories for solving problems. As such it has bonded closely with commercial industry. Many projects in analytical chemistry strive to increase the performance of any one system, in which performance is not only measured by the speed or accuracy of an analysis but also by other criteria, such as a system's ability to perform simultaneous and/or multiple sequential tasks. The project, at hand here, was to develop an analytical procedure for arsenic speciation analysis.

Throughout the duration of the project a few specific disciplines of analytical chemistry were amalgamated in search for a solution to the problem at hand. The disciplines included atomic spectrometry, liquid chromatography, and flow injection (FI) analysis. Using these tools of analytical chemistry we had set an objective to develop a method to determine the presence, in a water sample, of four environmentally significant arsenic species which are the inorganic trivalent arsenous acid (As(III)) and pentavalent arsenic acid (As(V)) and the methylated arsenic acids, which are monomethylarsonic acid (MMAA) and dimethylarsinic acid (DMAA). The final chosen hardware utilized for the arsenic speciation study was high performance liquid chromatography (HPLC), which used an ion exchange column, connected to a d. c. plasma - atomic emission spectrometer (DCP-AES). The

detection of arsenic species by the DCP-AES was an on-line process subsequent to the separation into individual species by HPLC. The theory of each analytical tool plus other information concerning arsenic speciation will be discussed during the rest of the thesis introduction.

1. Importance of Arsenic Speciation Studies

Arsenic present in the environment is the result of both anthropogenic and natural origins. The majority of industrial applications using arsenic compounds include pesticides, herbicides, and wood preservatives.[1] Most of the arsenic applications are now superseded by other compounds not containing arsenic. Interest in the presence of arsenic is accountable to the toxicity with which its history is associated. Though it is now recognized that arsenic compounds found in the environment occur in different chemical forms or species and exhibit complex environmental chemistry, the speciation of arsenic with respect to oxidation state and degree of methylation is important with regards to toxicity. The inorganic species are much more toxic than the organic species where As(III) is the most toxic of the four species. The toxicity of selected arsenic compounds are given in Table I.[2] The species listed in Table I are only a few of the total but are the most commonly analyzed. Therefore speciation analysis yields more useful information than does total arsenic analysis.

Studies performed strongly suggest that arsenic species found in aquatic environments

are predominantly the inorganic As(III) and As(V) plus MMAA and DMAA.[1] In 1973, Braman and Foreback[3] provided the first account of these four arsenicals in both marine and fresh water samples. All four species were found in each sample, though DMAA was almost always found in much larger concentrations than MMAA and ranged in concentration from 8-67 % of the total arsenic. Andreae, who is also a pioneer of arsenic speciation, observed similar concentrations ratios of the four species in both seawater and fresh water but also performed depth study analysis.[4,5,6] He concluded that the organic species' concentrations were strongly correlated with the photosynthetic activity of the depth. The organic species were only found in the photic zone and not in the deep waters below this region.

Other important arsenic species such as arsenobetaine and arsenocholine, which fall into the category of arsonium compounds, are essentially non-toxic. Arsenocholine was considered non-toxic because no effects were observed when fed to an animal as 1% of its diet.[7] Doses from 4-400 μg of As/g of body weight exhibited no signs of toxicity to rats, rabbits, and mice.[8] Arsonium compounds are poorly adsorbed by mammals and are rapidly eliminated through the urine.[9] These two species are not a priority with respect to the focus of this study because, to date, they have been found only in tissues samples of seawater organisms and not found in water samples.

Table I. Toxicity of selected arsenic compounds.

Arsenic Species	Chemical form	Toxicity*
trivalent arsenous acid, As(III)	$AsO(OH)$	20
pentavalent arsenic acid, As(V)	$AsO(OH)_3$	41
monomethylarsonic acid, MMAA	$CH_3AsO(OH)_2$	700-1800
dimethylarsinic acid, DMAA	$(CH_3)_2AsO(OH)$	700-2600
arsenobetaine, AB	$(CH_3)_3As^+CH_2CO_2^-$	Non-toxic†
arsenocholine, AC	$(CH_3)_3AsCH_2CH_2OH$	non-toxic‡
aspirin		1500[a]

*(the toxicity was measured as the 96h LD_{50} doses for rats in $mg \bullet kg^{-1}$)[2]
† ‡ non-toxic when fed as 1% of an animal's, which was unspecified, diet. These substances are considered non-toxic.
[a] BDH Material Safety Data Sheet: B27039

2. Atomic Emission Spectrometry

Atomic spectrometry is a tool for elemental analysis. It is concerned with the determination of the concentration of a particular element(s) in a sample irrespective of its chemical form. Spectrometric methods, though are very old, have only recently been applied to the determination of trace and ultra trace elements. Atomic emission, atomic fluorescence, and atomic absorption are examples of the physical phenomena that are utilized by modern spectrometric methods for elemental determination. Spectrometry is based upon the interaction of matter and radiation, in which the radiation can either be absorbed, emitted, and fluoresced and therefore is a technique dependent upon physical properties rather than chemical properties of the elements. More modern analytical methods such as mass spectrometry and nuclear magnetic resonance exhibit a similar trend towards dependency upon physical properties of the elements.

The two major categories of atomic spectroscopy are atomic absorption and atomic emission. These two phenomena are essentially reciprocals of each other and share many of the same principles. Electrons in the valence shell, which are also electrons with the least energy, are responsible for the physical process. The electrons are involved in transitions between energy states as a result of excitation by an external energy source. For ease of argument and applicability to this thesis only the atomic emission case will be further discussed since the spectrometry technique employed here was direct current plasma - atomic emission spectrometry (DCP-AES).

Atomic emission is the result of an electron 'relaxing' to a lower energy state from a higher energy state. The physical process is graphically depicted in a simplified manner below.

$$Ar^{+}+E^{0}\rightarrow E^{*}\rightarrow E^{0'}+h\nu \quad (1)$$

where Ar^{+} is an ionized Argon atom, E^{0} is an analyte in its ground state, E^{*} is the same analyte in an excited state, $E^{0'}$ is any lesser energy state than E^{*} of the analyte including the ground state, h is Planck's constant, and υ is the frequency of light emitted. The equation above depicting Ar^{+} is specific to the DCP-AES employed during this present study because the DCP reduces the analyte in the sample to their atomic form using a high temperature plasma in which the plasma is comprised of highly energized argon ions. In the first part of the above equation, the analyte enters the plasma and interacts with the highly energized Argon ions becoming excited to an arbitrary state, E^{*} . The excited analyte atom can undergo many different processes, one of which is relaxation. This event releases a photon equal in energy to the difference between energy states, E^{*} and $E^{0'}$, involved in the transition shown in equation 1. (Note that $E^{0'}$ could equal E^{0}.) The energy of the photon is E' and is shown in equation 2.

$$E'=E^{*}-E^{0'}=h\nu \quad (3)$$

$$h\nu=E_4-E_3 \quad (2)$$

E_4 and E_3 represent any arbitrary energy level that are not degenerate.

$$\nu = \frac{c}{\lambda} \qquad (4)$$

From equation 2 or equation 3, the wavelength characteristic to the transition of each element can be calculated as shown in equation 4. These equations are obviously very elementary. Other events frequently occurring in the plasma are as follows. The relaxing atoms can reabsorb emitted radiation or the analyte atoms can be ionized by the hot plasma. In some cases the emission intensity of the ionized analyte atoms are stronger than the primary resonance lines and as such are used for measurement.

The process is further described numerically by the Boltzmann Distribution relationship. The Maxwell - Boltzmann distribution given below describes the ideal situation governing emission.

$$\frac{N_1}{N_0} = \frac{g_0}{g_1} e^{-\frac{E}{kT}} \qquad (5)$$

where E is the energy between the electronic states involved, T is the temperature of the atom population, N_1 and N_0 are the atom population in the different states, g_0/g_1 is the statistical weights in the energy states 1 and 0, and k is the Boltzmann constant.

There are a few assumptions that must be stated initially. First of all, it assumes that

the population of atoms are in thermal equilibrium at a constant temperature, T. Secondly, the energy term, E, applies to all electronic energy terms: vibrational, rotational, and translational. This relationship allows us to calculate the population of atoms that can be excited by the plasma knowing that the thermal temperature of the system is related to the translational energy of the atoms. Even though the electronic temperature and thermal temperature are different physical properties usually no distinction is made between the two during generalized discussions.

When an atom is heated, the number of electrons in any particular orbital can be calculated by the Boltzmann distribution. Also from the distribution, it can be seen that the relationship between energy states is dependent upon the temperature of the system. Now, as it applies to emission spectroscopy, the equation allows us to calculate the number of electrons in excited states: higher temperatures of the system results in an increased number of electrons in elevated energy states. Therefore, atomic emission is most effective at very high temperatures for reasons already mentioned and other reasons which will be discussed below. A disadvantage of emission spectroscopy is that higher energy transitions corresponding to shorter wavelengths will have lower emission intensities than those transitions at longer wavelength whereas atomic absorption measurements are independent of wavelength. Atomic absorption is not mathematically governed by either the temperature or the energy of transition, E, and therefore wavelength, which is a function of the energy of transition (i.e. emission intensity), does not factor into the degree of absorption. Atomic absorption is governed by the Beer-Lambert law.

$$A = abc \tag{6}$$

where A is absorbance; a is the absorption coefficient; b is the path length through the sample; and c is sample concentration. The disadvantage experienced by atomic emission is somewhat nullified by the extreme temperatures used in plasma emission because of the significantly large fraction of atoms in the excited state.

Plasma energy source instruments are very effective tools for atomic emission spectroscopy. The excitation source, which is referred to as the plasma torch, is very hot and stable. Temperatures of the plasma are often estimated as 5000-15000 K. These electronic temperatures are calculated by measuring the emission intensity, S, of the plasma. The plasma emission is measured at the wavelength where its intensity is a maximum. Where,

$$S = N_1 \frac{E}{\tau} \tag{7}$$

$$S = \frac{N_0 E}{\tau} \frac{g_1}{g_0} e^{-\frac{E}{kT}} \tag{8}$$

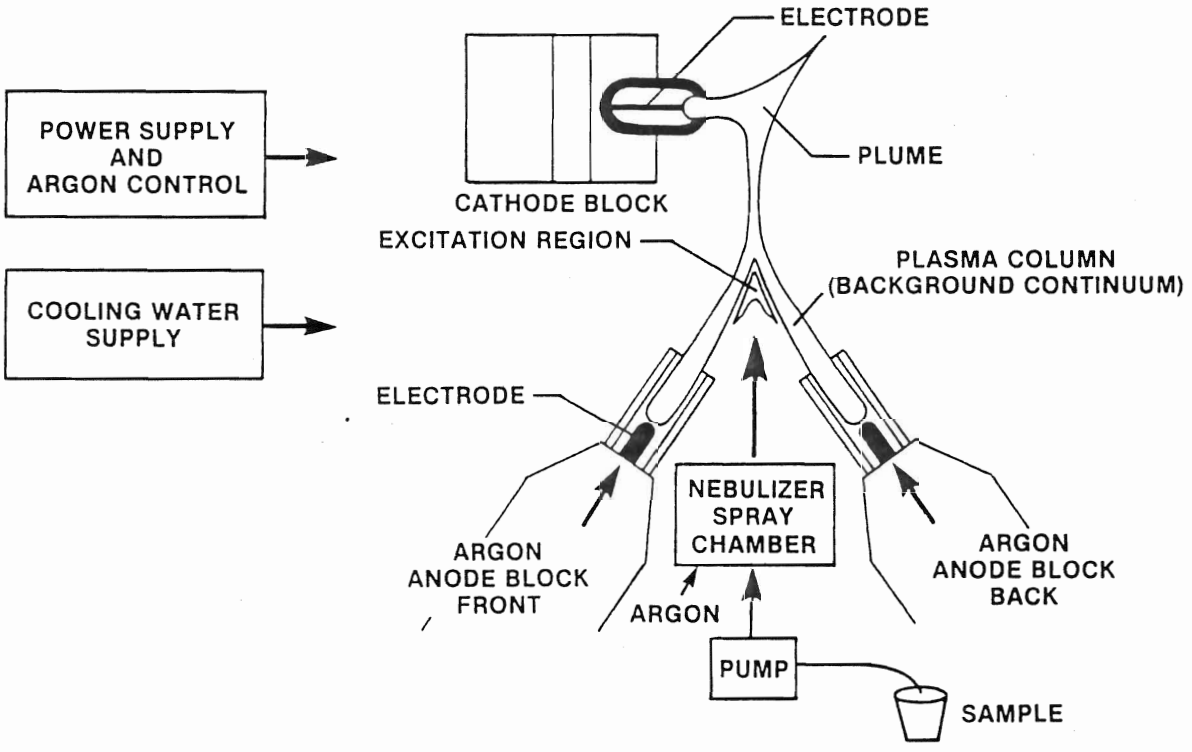
the energy of the excitation is calculated by measuring the frequency, ν, of radiation emitted and using equation 1; τ is defined as the lifetime of the atom in the excited state; T is the temperature of the system; and k is the Boltzmann constant. The direct relationship between

temperature and the number of atoms in excited states is illustrated here. At these extremely hot temperatures the plasma is capable of atomizing and exciting all metals and metalloids in the Periodic Table. The above discussion was derived from the book *Atomic Spectroscopy*, written by James W. Robinson, who, along with Sir Alan Walsh, was one of the founders of atomic absorption spectrometry.[10]

Direct Current Plasma - Atomic Emission Spectrometer

There are three commonly used plasma types: the inductively coupled plasma (ICP), or RF plasma, the DCP and microwave-induced plasma. All three types are modern analytical tools with commercial introduction of the ICP and DCP in the early 1970's. Due to the relevance to this thesis, only the DCP will be discussed. First, a brief historical account of the development of the DCP.[10] The DCP was first introduced by Margoshes and Scribner.[11] Since then the instrument has had a short series of modifications leading to the design used here. The commercially renowned DCP was originally developed by Valente and Schrenke.[11] In 1974, it was modified by Elliot, who added a third electrode. Finally, SpectraMetrics Inc., U.S.A. commercialized the familiar inverted 'Y' plasmajet.[12] The inverted Y shape of the plasmajet is the result of two separate d.c. arc plasma jet sources: two pyrolytic graphite anodes with a common tungsten cathode. The schematic representation of the electrode configuration and plasmajet is shown in Figure 1.[13]

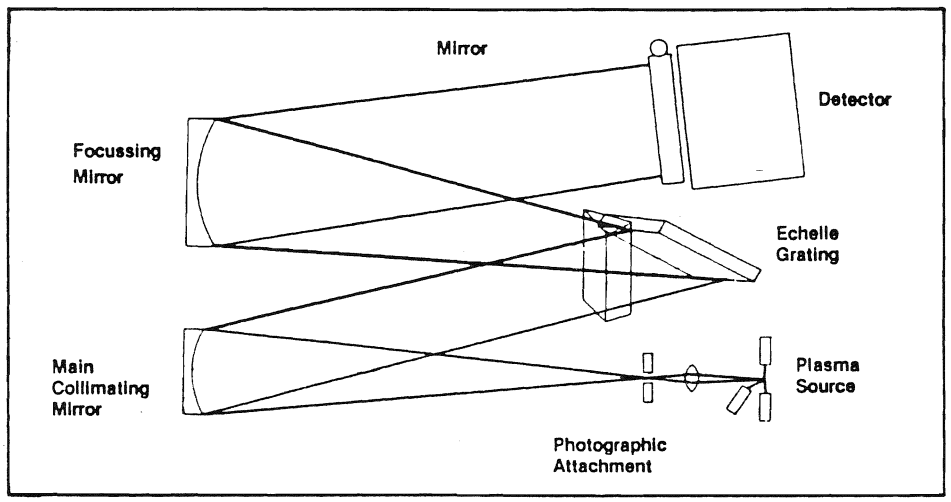
Figure 1. Schematic representation of the D.C. plasmajet and the electrode configuration.



Three main components of the DCP-AES instrument include: the plasma, the optics, and the detector. The plasma is a system of highly energized and ionized argon gas atoms. The operation of the plasma is relatively simple and includes the previously mentioned information. The plasma is initially ignited by shorting the anodes with the cathode then all the electrodes all 'pulled back' generating and maintaining an argon arc. The electrodes are cooled by the flowing argon through the electrode cavity and by circulating cooling water in the electrode jacket. Operating temperatures of the d.c. plasma are approximately 5000-10000 K. The measured excitation temperature of the region below the junction of the two arcs was 6000 K.[14] This area below the plasma is used for emission measurement because it is hot enough to atomize the analyte with low background intensity from the argon plasma as compared to a region within the plasma arc.

The optical system of the DCP-AES is an Echelle grating based spectrometer and is graphically presented in Figure 2.[15] The layout of the system is a Czerny-Turner configuration but the inclusion of an Echelle grating allows for a compact design while maintaining high resolution. The optical system is important because it resolves the emissions of the elements in the sample into individual wavelengths. From the range of radiation emitted by the analyte, only characteristic wavelength(s) are selected for measurement. The wavelength(s) selected is a physical property unique to that element being measured in the sample. The Echelle spectrometer resolves the incident radiation using a grating and a prism in tandem, and in that order. Both components disperse the radiation.

Figure 2. Optics design for the SpectraSpan Echelle spectrometer.



Optical Schematic

The prism refracts and disperses the light in one plane. The grating disperses the light in one polarized plane, then this polarized light is incident upon the prism which refracts and disperses the light at right angles to the plane of the grating. The result is a two-dimensional array of resolved light. In the Echelle spectrometer, the grating is rotated such that the wavelength of light desired is selected and directed using mirrors towards the detector.

The detector within the DCP is a photomultiplier tube (PMT). The PMT is a vacuum tube which contains a photosensitive cathode and a series of anodes called dynodes. The PMT operates as follows. A photon of light (or packet of photons) strikes the cathode releasing electrons. The electrons are accelerated towards a slightly more positive potential dynode. Each successive dynode, along the path travelled by the electrons, is slightly more positive in potential forming a potential ramp to power the electrons. Electrons striking each successive dynode release even more electrons called secondary electrons. The process repeats itself until the electrons reach the anode. The charge of the anode is now large enough to measure with conventional electronic equipment. A typical PMT has 8-10 dynode stages. This event lasts only nanoseconds and releases millions of electrons during each photon event. Other detectors used with atomic spectrometry are mass spectrometers[16,17] and photo diode arrays[18].

3. Sample Introduction

The conventional method of introducing the analyte into the plasma is by

nebulisation. This DCP uses a pneumatic crossflow nebulizer which aspirates liquid solutions into the plasma. A peristaltic pump feeds the liquid sample into a junction at the point where the carrier gas, argon in this case, is forced through a nozzle. A fine mist is formed in the immediate expansion chamber which is carried to the plasma. Unfortunately, no more than 10% of the liquid sample is transported to the plasma.[19] The rest form droplets which fall out of the carrier stream and go into the waste. The aerosol that does reach the plasma must contain a small range of drop sizes because droplet size range influences the limit of detection and the coinciding standard deviation of precision for a particular analyte.[20] Size of the droplets were estimated at 3-10 μm and 1-20 μm.[12] Smaller droplets of a uniform size are desirable because they result in a more stable plasma which in turn decrease signal noise and increase measurement precision.[21] In summary, disadvantages of liquid aspiration include inadequate control of drop size, turbulent carrier gas flows, and poor analyte transport efficiency. Therefore replacement of inefficient conventional nebulization is necessary and desirable. Possible alternatives are vapour phase introduction via hydride generation or electrothermal vaporization, ultrasonic nebulization, direct sample insertion (DSI)[22] and direct injection nebulization (DIN).[23] This latter device uses a modified nozzle, which is an integral part of the DIN, situated immediately before the plasma. The DIN delivers significantly reduced volumes of 15% of 120 $\mu l \bullet min^{-1}$ or on the order of 15-30 μl after splitting of the original sample.[24] Unfortunately the device, which is manufactured by Ames Laboratory Inc., U.S.A., is both very expensive and must split the liquid sample before introduction for total sample sizes above 100 $\mu l \bullet min^{-1}$.

Hydride Generation

Hydride generation (HG) is an advantageous replacement to conventional nebulization. During hydride generation the covalent volatile hydrides of the analyte are produced by reaction with a reducing agent such as sodium tetrahydroborate(III), thereby allowing gaseous sample transport to the plasma and in turn eliminating the use of the nebulizer. The analyte can easily be transported in amounts rivalling 100% efficiency. The hydride formation also increases efficiency by eliminating the need for the plasma to desolvate and vaporize the mist into a dry analyte aerosol resulting from liquid aspiration. There are extensive studies being currently pursued in other laboratories to increase sensitivity during sample introduction by pre-evaporating the aerosol.[25] Therefore sample introduction of the volatile hydrides significantly betters the detection limit over conventional nebulization because it separates and preconcentrates the analyte from the liquid sample. Preconcentration occurs as a result of rapid introduction of the entire analyte sample from the sample solution which would require a much longer time by nebulization. For instance, detection limits using hydride generation are improved by approximately a factor of 20 over liquid nebulization.[12]

Hydride generation was first introduced by Holak[26] in 1969 and has since been used for the trace analysis of many metalloid elements. Since then numerous papers report using hydride generation during the analysis. Ideally, during hydride generation, the analyte will form the volatile hydride and be carried out of the sample devoid of any matrix effects. A

major disadvantage, though, is the interference caused by other elements that form hydrides. In addition, interelement interferences are produced by easily reduced transition metals. Some transition metals commonly found producing interferences are nickel(II), copper(II), and cobalt(II).[27]

Metalloid elements that most benefit during trace analysis by forming hydrides are from groups IVA, VA, and VIA in the periodic table. A small list of studies demonstrating this technique with various elements include antimony[28,29], arsenic, bismuth[28], germanium[30], mercury[31], selenium[32,33], tellurium[34] and tin[35]. It is important to note that production of hydrides is dependent on the oxidation state of the atom. For example, trivalent arsenic forms the hydride more easily (under less harsh conditions) than does the pentavalent arsenic. The As(III) produces the hydride in low acid concentration solutions at pH 1-4 whereas As(V) produces the hydride only in concentrated acid solutions of 4-6 molar.[36] Almost all methods reported using sodium tetrahydroborate(III), stabilized in dilute hydroxide, as the standard reducing reagent to produce the hydride. The hydride reaction with As(III) is shown below.

$$HCl + 3H_2O + NaBH_4 \rightarrow H_3BO_3 + NaCl + 8H^- \quad \textbf{(9)}$$

$$xH^- + As^{III} \rightarrow AsH_3(g) + H_2(g) \quad \textbf{(10)}$$

To date, the conventional determination of arsenic using hydride generation has generally reported a total arsenic content of the sample.[37,38,39,40] Thompson and Thomerson,

1974, provide the first account of the conditions required for efficient reduction of arsenic compounds to their corresponding arsines.[41] Two common methods for inorganic arsenic determination by hydride generation consist of either a concentrated hydrochloric acid to measure As(III) + As(V) or a pH controlled reaction generation medium, which allows the analyst to differentiate between As(III) and As(V).[3,38] The pH of the solution was maintained between 4 and 5 for the selective determination of As(III). Another technique to measure total arsenic content uses concentrated HCl to which KI is added as a prereductant for the pentavalent arsenic species.[42,43,44,45] Sometimes ascorbic acid is added to the reducing agent in order to prevent the oxidation of I^- to I_3^-.[43,45] Addition of ascorbic acid is necessary to prevent iodine contamination of equipment and to reduce interferences in atomic absorption (AA) signal sensitivity. Unfortunately, iodide is an effective prereducing agent only in concentrated acid media, at a pH of less than 1.

An early arsenic speciation scheme was reported by Howard and Arbab-Zavar in 1981.[44,46] The authors report a technique for the selective determination of the inorganic species, As(III) and As(V), plus the organic species, MMAA and DMAA , by cold trapping all arsines followed by controlled heating of the atomiser in the AA to determine the amounts of each species in a sequential manner. Sturgeon *et al.*[47] recently published a very good speciation study about selective volatisation after cold trapping the arsines. The authors suggest that other volatile arsenic species may be sequestered and, as such, the technique is suitable for total As only. Hinners offers a different warning.[45] During simultaneous measurement by direct hydride introduction (i.e. no cold trap), which utilises inorganic

arsenic standards, the results can be misleading because of interferences from organic arsenic species. These interferences were of an enhancing nature. The assessment of selective determination by gas-phase volatization after cold trapping is furthered later in the introduction.

Finally, another method uses a microwave digestion procedure with potassium peroxodisulphate to decompose and oxidize the arsenic analytes.[37,48] The arsenicals after the digestion pretreatment exist entirely as As(V) and are measured using a concentrated acid during HG.

Two very recent studies, which were both published in 1992, report the determination of arsenic species also using media with the pH significantly below 1. Lopez *et al.*[49] reported that the generation of arsine from both of the inorganic arsenicals could be performed without KI provided that the flow rates of $NaBH_4$ and Ar carrier gas were controlled in order to compensate for the kinetic differences of arsine generation between the two species. The authors claim that arsine can be carefully collected by slowly stripping the arsine from the solution. Selective arsine generation from As(III) was carried out in a citric acid/citrate buffer at pH 4.5 whereas total arsenic content was determined in 2 M HCl. The interference effects from the organic arsenicals were also studied. The chemical method can be used for As(III) and As(V) speciation when DMAA and MMAA concentration does not exceed 40% total arsenic, otherwise the methylated species enhance the signal. Unfortunately, this technique does not lend itself to a rapid on-line detection method scheme.

In the second paper, Murer *et al.*[50] report a method for the determination of As(III), As(V), MMAA, and DMAA by hydride generation in urine using FI-AAS. Spiked samples of As(III), MMAA, and DMAA had a recovery not significantly different from 100%, whereas As(V) recovery was reduced. The authors here conclude that the reaction solution must have a pH below 1 to obtain optimum hydride generation but the continued use of high acid concentration is disadvantageous because of its detriment to equipment and higher cost of operation expenditures as compared to much lower acid concentrations.

Several other published studies were aimed at examining the effects of various reducing media on arsenic containing compounds. Ebdon *et al.*[51] investigated the formation of the stable arsenic derivatives with methylthioglycolate and commented on the reduction of arsenate to arsenite in dimethylarsinic acid (DMAA) and monomethylarsonic acid (MMAA) during their derivatization. Cullen *et al.*[52,53] investigated the reduction of methylarsenicals with an assortment of thiols. The work performed, though not directed at hydride generation, was used to study the reactions between thiol compounds and organoarsenic compounds for characterization of arsenic during biological transformations. DMAA, MMAA, and trimethylarsine oxide are easily reduced by thiols, such as cysteine, glutathione, and lipoic acid, to give the corresponding organosulfur derivatives.

A series of publications by Anderson *et al.*[54,55] studied hydride generation of the four arsenic species in numerous solution media plus the effects of various reagents with regards to metal interferences. These publications were very informative to the scope of this thesis.

One of these publications provides a summary of redox systems used in other publications for altering the oxidation state of As(III) and As(V).[54] Selective reduction in different reaction media allows for the determination of the arsenic species both individually and in combination with others. The results were as follows.

(a) As(III) was best determined individually in a potassium hydrogen phthalate buffered medium or citric acid - sodium citrate buffer.

(b) Potassium iodide (0.1%(m/V)) in 5 M HCl allowed the selective reduction of As(III) + As(V) with minimal interference from MMAA and negligible interference from DMAA.

(c) DMAA alone was determined in the following two media. A matrix containing acetic acid plus potassium permanganate (0.5 $\mu g\ mL^{-1}$) was added to oxidize inorganic arsenite to arsenate and thus negate enhancing interferences from As(III). The second medium used to determine DMAA alone was a matrix containing potassium iodide in dilute acid (e.g. 0.3 M HCl, 0.16 M AcOH). More severe interferences from other arsenic species were found in the permanganate matrix.

(d) In a citric, oxalic, or acetic acid medium, As(III) + DMAA were selectively reduced and produced similar strength signals which were much stronger than As(V) and MMAA.

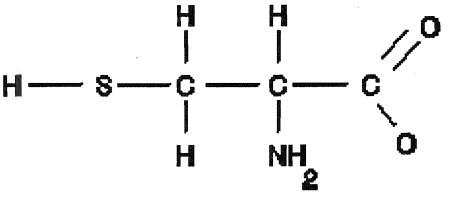
(e) A mercaptoacetic acid matrix (0.1 M) effectively reduced As(V), MMAA, DMAA and gave a 'total' arsenic measurement of all four species (As(III) + As(V) + MMAA + DMAA).

Mercaptoacetic acid, also referred to as thioglycolic acid ($HSCH_2COOH$), is a sulphydryl containing ligand (i.e. thiol) and the results observed using it are most relevant to this thesis. The studies of Anderson *et al.*[54], Ebdon *et al.*[51], and Cullen *et al.*[52,53] and the citations therein have proven that thiol containing compounds have the ability to reduce arsenate to arsenite in both inorganic and organic species. Therefore this important result was applied to work in a hydride generation technique because it is well understood that the efficiency of the arsine generation is controlled by the oxidation state and degree of methylation of the arsenical. We had proposed that L-cysteine, which is also a thiol containing compound, would have a similar effect as mercaptoacetic acid and produce similar sensitivities for all four arsenicals during HG. At the onset of this study there were some preliminary advantages of a cysteine reagent over mercaptoacetic acid. First of all, Cullen *et al.*[52] demonstrated that cysteine was more effective than mercaptoacetic acid at reducing both methylated arsenic species. The authors report percent yields for reactions between DMAA and MMAA with cysteine of 25% and 13%, respectively, and with mercaptoacetic acid of 6% and 0%, respectively. Another advantage, with regards to the operator, was that cysteine has a very slight odour as compared to the pungent odour of mercaptoacetic acid.

Hydride generation as a means of gas phase sample introduction for AES has been studied extensively by I.D. Brindle and co-workers.[56,57,58] Trace analysis has focussed on the elements germanium, tin[59] and arsenic. It was discovered that the use of thiol containing compounds as prereducing agents allowed for identical, less harsh conditions regardless of the oxidation state of the metalloid. Compounds such as L-cystine and L-cysteine

significantly reduced the interferences from transition elements. L-cysteine, though L-cystine was better for reducing interferences, was chosen from the group of thiols as the best suited for inorganic arsenic determinations. The chemical formula for L-cysteine is shown below.

L-cysteine is also commonly referred to as cysteine; its IUPAC abbreviation is Cys; β-mercaptoalanine; 2-amino-3-mercaptopropanoic acid; 2-amino-3-mercaptopropionic acid; and α-amino-β-thiolpropionic acid. It is formed from the cleavage of proteins during hydrolysis. There are no extraordinary safety precautions that need to be followed when handling L-cysteine whereas there are extra precautions must be undertaken when using thioglycolic acid (mercaptoacetic acid) because contact can cause severe burns and blistering of skin in addition to its strong unpleasant odour.



In conclusion, advantages of using L-cysteine reagent during arsine generation are as follows:

(1) L-cysteine can efficiently reduce As(V) to As(III) in low acid concentration and enhance signal sensitivity.

(2) Hydride generation in low acid concentration substantially reduces hydrogen gas evolution therefore increasing the stability of the plasma in the DCP.

(3) The low toxicity of the reducing reagent and the reaction medium are favourable to the operator.

(4) Contamination and corrosion of the equipment is reduced using low acid concentration.

4. High Performance Liquid Chromatography

Chromatography, in general, is the science of separating the desired components of a sample with either quantitative or qualitative identification. A particular type of chromatography is High Performance Liquid Chromatography (HPLC) and separation of the analytes, which exist as solutes, is the result of a dynamic differential migration between a finely divided stationary phase and a mobile phase used to transport the solutes. The mobile phase is a continuously flowing stream forced through the stationary phase under high pressure in which the solutes exhibit differing mobilities based primarily upon differences in adsorption and solubility within the two phases. These events occur in a device called a 'column' which houses the stationary phase and range in length from 100 to 300 mm with an internal diameter less than 1 cm. The stationary phase can exist as liquid films adsorbed to a solid phase or as a specific functional group chemically bonded to an inert substrate. Commonly available substrates are silica and the poly(styrene-divinylbenzene) resin.

Desirable properties of the substrate is that it must have a very high surface area to volume ratio in order to assure maximum contact between the mobile and stationary phases. As such, the diameter of stationary phase particles are on the order of microns. It must also avoid swelling or shrinking regardless of the mobile phase and must also withstand various media.[60]

HPLC contains many classifications. Commonly used methods, for instance, are normal-phase, reversed-phase, ion-pair reversed-phase, ion exchange, gel filtration, or gel permeation. Ion exchange chromatography (IC) was the method of choice in this study and therefore will be the focus of the discussion. The reasoning behind choosing IC will be discussed in the discussion section.

IC columns contain insoluble porous beads having cationic or anionic sites at the surface that can exchange anions or cations from the mobile phase. The beads are comprised of either a silica or polymeric substrate onto which are attached covalently bonded dissociable functional groups. Sulfonic acid functional groups are used for cation exchange and either tertiary amino or quaternary ammonium groups are used for anion exchange.

Separation of the compounds in all types of HPLC occurs because differences in physical properties of the components produce different equilibrium distributions of the components between the stationary phase and the mobile phase.

$$X_m \rightleftharpoons X_s \quad (11)$$

where X_m is the analyte molecule in the mobile phase and X_s is the analyte molecule when interacting with the stationary phase. The components migrate through the column at different rates depending upon their interaction with the stationary phase. Interaction with a solid stationary phase is referred to as adsorption. The more strongly a substance adsorbs to the solid the slower is its progress through the column. Similarly in IC, separation is based on the affinity differences between the analyte ions and eluent ions towards their counter-ions in the ion exchange matrix.

$$X_m^{n-} + nR^+M^- \rightleftharpoons nM_m^- + [R^+]X^{n-} \quad (12)$$

where X is the analyte anion in the sample, M is the mobile phase anion, and R is the anionic functional group bonded to the stationary phase. The mobile phase typically contains a salt which, in addition but not necessarily, may have buffering capacities.

Fundamental Physical Processes of IC

Chromatographic separations are the result of very complex interactions between the solute, the eluent, and column packing. Physical properties of the eluent such as flow rate, ionic strength, and pH, combine with stationary phase properties: size, packing configuration,

support type, and functional group during the chromatographic separation and not to mention chemical processes. Simplified models are described below.

We have stated that chromatography is basically an equilibrium process in equation (10). Therefore a general performance measure of the column describing its efficiency is the column platenumber, N, and is based on the number of equilibrium stages obtainable in the column.

$$N = \left[\frac{t_R}{\sigma_t}\right]^2 = 16\left[\frac{t_R}{w}\right]^2 \qquad (13)$$

where t_R is the retention time by isocratic elution, σ_t is standard deviation of the peak in time units, w is the peak width, which is measured as the distance on the baseline between the tangents of the inflection points (assuming Gaussian shape peaks; $w=4\sigma$).[61] Typical platenumber for modern columns ranges from 1000-10000. Related to platenumber is plateheight, H, which is commonly referred to as Height Equivalent Theoretical Plate (HETP).

$$H = \frac{L}{N} \qquad (14)$$

where L is column length and N is the column platenumber.

Retention time of the analyte in the column is measured as the time the analyte spends in the column minus the column deadtime. Column deadtime is the time required for

$$t_R = t_0 + t_R' \quad (15)$$

a solute that does not interact with the stationary phase to pass through the column. where t_0 is the column deadtime; t_R is the gross elution time of the analyte; and t_R' is the elution time of the analyte beyond the column deadtime.

Another measure of column efficiency is k'. This value has many names such as mass distribution ratio, capacity factor, distribution ratio, etc.

$$k' = \frac{[\text{analyte(stationary-phase)}]}{[\text{analyte(mobile-phase)}]} \quad (16)$$

$$k' = \frac{t_R'}{t_o} \quad (17)$$

Analytes in IC undergo acid/base equilibria very rapidly. The analyte only interacts with the stationary phase when it is ionized therefore it is important to understand the extent to which it is ionized.

$$AH \rightleftharpoons A^- + H^+ \quad (18)$$

$$f^- = \frac{1}{1 + \frac{[H^+]}{K_a}} \quad (19)$$

where f^- is the fraction ionized; $[H^+]$ is acid concentration; and K_a is the acid dissociation constant. After some manipulation, the capacity factor in IC can be described as:[62]

$$k' = (\text{constant}) * f^- * c_b^{-n} \quad (20)$$

where c_b is the concentration of the buffer in the eluent. Therefore it is shown that separation in IC is dependent upon the net charge and degree of ionization (pK_a value) of the analyte, the ionic strength of the eluent, plus other factors not mentioned. For instance, multiply charged ions are bound more than singly charged ions. Binding strengths fall into the order $M^{4-} > M^{3-} > M^{2-} > M^{1-}$, therefore As(III) should elute before As(V).

Resolution is the differentiation between adjacent peaks in a chromatogram and is most often the measure studied during analysis. Resolution, R, is expressed in terms of the difference in the retention times of the two components, t_{R2} and t_{R1}, and the arithmetic mean of the two bandwidths, w_1 and w_2, which are measured at the base of the peaks. The equation for resolution is shown below in equation (21).

$$R = \frac{2(t_{R_2} - t_{R_1})}{w_1 + w_2} \quad (21)$$

Ideal chromatographic separations have baseline resolution between adjacent peaks with narrow peak widths and sharp, Guassian peak shapes which indicate efficient interaction with the stationary phase and a minimum of longitudinal diffusion. In many circumstances compromises must be made between resolution and peak shape. Resolution factors of 1.5 will suffice when the peaks are Gaussian shape. A gradient elution program of the mobile phase may be necessary when there exists a large difference between the retention times of the analytes in order to maximize the peak height and to minimize the time required for each analysis. For example, the separation of acids with different pK values can be achieved with a pH gradient elution.[63]

Arsenic Speciation Analysis using Chromatography

As mentioned previously, there is an emphasized interest into arsenic speciation because a speciation determination provides much more useful information than does a total arsenic determination. Liquid chromatography is one such tool for a speciation scheme. Gas chromatography (GC) is another method for arsenic speciation. There are two different GC-type methods used for speciation. One GC method is a conventional GC method that separates the analytes using a packed column containing a stationary phase. The second GC method resolves the analytes by selective volatisation of gaseous arsine species without the use of a stationary phase-packed column. A brief description of this latter method is as follows. The first step in this technique involves the formation of the gaseous arsine from all inorganic and organic species. The analytes are collected in a liquid nitrogen cooled trap.

The trap is then carefully heated and the species are separated by the difference in boiling points. The different boiling points are shown in Table II. There are several published studies that use GC for arsenic speciation and are extensively reviewed by Ebdon *et al.*[64]

GC, along with LC, was an original method for the speciation of arsenic and it was first demonstrated in 1975 by Talmi *et al.*[65,66] and in 1977 by Braman *et al.*[67] In a recent study aimed at optimizing the parameters using GC-AAS, each analysis required 200 s, which was extended to 600 s for As(III) determination, for collection of the arsines plus 300 s for the final heating of the cold trap.[68] Although this method is an attractive alternative to LC separation, one major disadvantage is the inability to determine As(III) and As(V) in a single run since these arsenicals produce the identical arsine. A separate pH buffered analysis must be run to selectively determine As(III).

Short Summary of Published HPLC Techniques for Arsenic Speciation

There are a number of published reports on arsenic speciation using HPLC interfaced with atomic spectrometry. The list of methods and detection limits are given in Table III. From the data presented, detection limits were dependent upon the detector used and worsen in the order ICP-MS, GFAAS, and ICP-AES. In one report, the authors compare AAS and

Table II. Hydride form and boiling points for four arsine species.

Species	Hydride form	boiling point [67](°C)
As(III)	AsH_3	-55
As(V)	AsH_3	-55
MMAA	CH_3AsH_2	2
DMAA	$(CH_3)_2AsH$	35.6

Table III. Evaluation of published HPLC techniques for arsenic speciation.

Ref#	chromat. method	detector	sample intro.	elution t (min)	equil t (min)	abs. d.l. (ng)
69	IC	ICP-MS	nebulizer	5	n/a	0.017-0.035
70	IC	AAS	HG	10	n/a	0.2
71	RP	AAS	HG	3	2	0.05-0.47
72	RP	ICP-AES	nebulizer	14	n/a	13
73	IC	ICP-AES	HG	7	n/a	3-25
73*	IC	ICP-AES	HG	n/a	n/a	10-21
74	IC	AAS	HG	3	n/a	2
75	RP	ICP-MS	TS	15	n/a	30-50
75	IC	ICP-MS	TS	40	n/a	20
76	IC	ICP-MS	nebulizer	10	n/a	0.02-0.09
77	RP	ICP-MS	nebulizer	9	n/a	0.05-0.30
78	IC	ICP-AES	nebulizer	15	n/a	2.6
77*	RP	ICP-AES	nebulizer	n/a	n/a	3.2-13

Ref#	chromat. method	detector	sample intro.	elution t (min)	equil t (min)	abs. d.l. (ng)
77*	n/a	ICP-MS	n/a	n/a	n/a	0.1-0.2
50	IC	ICP-AES	HG	60	n/a	20
50	IC	AAS	HG	60	n/a	0.6
79	RP	ICP-MS	DIN	30	n/a	0.0006
79‡	RP	ICP-MS	DIN	15	n/a	0.0002
this study†	IC	DCP-AES	HG	12	7	2500

Ion chromatography, IC; reverse-phase chromatography, RP; direct injection nebulizer, DIN; thermospray, TS

*refers to information obtained from references therein.

†the arsenic speciation analysis has not been yet calibrated for a detection limit. The concentration noted was used for most experiments.

‡second, of two techniques performed but used a step gradient during elution, compared to no gradient used in the other technique..

ICP-AES directly and found that AAS is 35X more sensitive than ICP-AES.[50] An assortment of sample introduction techniques are also shown but it is difficult to base any conclusions for the preference of any type from the data presented. Elution time in the table refers to the time required by the chromatography of the sample while equilibration time refers to any time required beyond the time to inject the next sample after the finish of the last chromatogram and the start of the next chromatographic run. Often chromatographic runs are made immediately after one another during isocratic elution programs.

Applications that link an HPLC procedure to a detector for analyte measurement during an on-line analysis are referred to as flow injection (FI) analysis systems. The discussion immediately below, in section 5, provides more information about FI analysis. It is widely accepted that HPLC, in essence, is an extension of FI techniques and in many cases can not be easily differentiated.[80] In certain situations, the experts have noted that the definition of FI tends to precariously merge into the definition of its predecessor HPLC and therefore it is important not to redefine an old technique but to allow each technique to maintain its integrity.

In short, HPLC lends itself to an FI technique because it is a particular means of injecting a sample of analytes into a carrier stream in which the purpose of HPLC is the prior separation of the analytes by the column before other on-line chemical reactions and/or manipulations. Finally, the present author suggests the reading of a review written by Ebdon

et al.[81] because this publication thoroughly covers techniques of liquid chromatography coupled to atomic spectrometry.

5. Flow Injection Analysis

Many definitions for FI have been formulated and this is indicative that FI is an extremely diverse technique which encompasses many types of analyses and various types of hardware used to perform the analyses.[82] FI analysis is very simply defined as the analysis of injections into a flowing stream. In the study at hand, FI analysis is descriptively described as the insertion of a discrete analyte slug into an unsegmented carrier stream inside narrow-bore-tubing and the measurement at the detector downstream. Although flow of the analyte is very laminar and requiring a minimum of volume, McLeod[16] observed that dispersion still occurs to varying extent and can be used in a positive sense because dispersions ideally produce Guassian shaped peaks which can be easily integrated. Therefore, once inside the stream, the analyte and reagents mix, by convective or diffusive forces, reacting to a desired product which will then be measured.[83,84] In some cases, a higher degree of mixing is required which can be obtained either by the FI manifold design or by forcing the flow through separate mixing chambers such as resin packed tubing. In other cases, mixing or diffusion of the analyte is minimized by gas-segmenting the carrier stream which is done by inserting small bubbles into the carrier stream.[96]

All signals in a FI analysis are transient, which means the signal is not steady-state, and the shape of the transient peak represents the concentration gradient of the analyte in the carrier stream. It is normal practice to relate the maximum peak height and/or the area under peak to the concentration of the original, undispersed analyte sample before injection. Once the FI system is developed to an appropriate degree, there is often work performed towards automating the system for reasons such as increased productivity and decreased labour requirements. A FI analysis can be easily automated because the system is purely repetitive, consistent and self-sustaining.

Many hydride generation methods incorporate a FI system as a means of transferring the analyte from a reaction manifold to the detector. Two common techniques of FI for HG are batch and continuous FI, though many variations exist.

Batch FI Analysis

Batch FI analysis, as its name suggests, deals with signal generation in a batchwise process. In our case involving hydride generation, the carrier stream is argon gas that passes through the reaction chamber and transports the evolved hydrides to the DCP-AES. The argon gas is also used to produce the plasma and aids in the mixing of reagents. The FI manifold is the reactor where the arsenic species mix with sodium tetrahydroborate (III) and the hydride is produced. Only a single measurement is made for each sample preparation and the entire sample preparation procedure which includes flushing, cleaning, and preparing the reaction cell must be repeated for every measurement.

In a batch system the volume of the analyte sample is often millilitres, as is the case in this study where the sample volume was 5.0 mL, whereas microlitre samples are used in continuous FI. This difference can have either a positive or negative aspect. On the negative side more sample is required for every determination which may be from a limited total amount, but on the positive side the absolute amount of analyte transferred to the detector is increased which will improve detection limits. Advantages of the batch system include strict control of the reaction conditions, such as pH and reagent concentrations, and reduced diffusion of the analyte because all of the workup required by the analyte, prior to sample introduction into the detector, is performed in a single, fixed-volume chamber. Therefore sample introduction by the batchwise process permits a more concentrated analyte plug to reach the detection system, as compared to a continuous FI system in which the analyte plug is dispersed as it travels through the mixing tubes along the FI scheme. Disadvantages of the batch are the disruption of the baseline signal with the introduction of the analyte, large volumes of analyte are required, the difficulty of applying to an on-line detection method, and the procedure is labour intensive.

Continuous FI Analysis

Many circumstances in analytical chemistry entail the coupling of instruments. Continuous FI analysis can couple the detector to: (a) pre-treatment apparatus, for: (i) preconcentration[16], and (ii) element speciation[79]; and (b) on-line processes, including: (i) HG, (ii) matrix modification (demonstrated in this study by the immediate addition of nitric acid

to the HPLC effluent), and (iii) chemical derivitization.[85] A continuous FI method is advantageous for the following reasons. Firstly, it enables a rapid speciation scheme in a simplistic manner. For instance in this case, certain species of an analyte can be separated using HPLC with subsequent additions of reagents to the effluent using a peristaltic pump. This is then followed by a reaction in a continuous hydride generator and finally, insertion into the argon carrier stream to the detector. This is an example of the many steps and processes that can be performed by a single FI system. Secondly, continuous FI is much less labour intensive to run than a batch system. Lastly, it is easier to automate than a batch system because, as already mentioned, once the system is running it is self-sustaining and usually requires fewer dynamic operations, excluding pumping actions, than batch FI. As well, automation of a batch system often necessitates the operation of solenoids, valves, and other motors which are prone to failure. On the other hand, major concerns of continuous FI techniques include the stability of the detector over extended periods, preparation of sufficient reagents, and the integrity of the entire system.

METHOD

1. Batchwise Hydride Generation of Methylated Arsenic Species

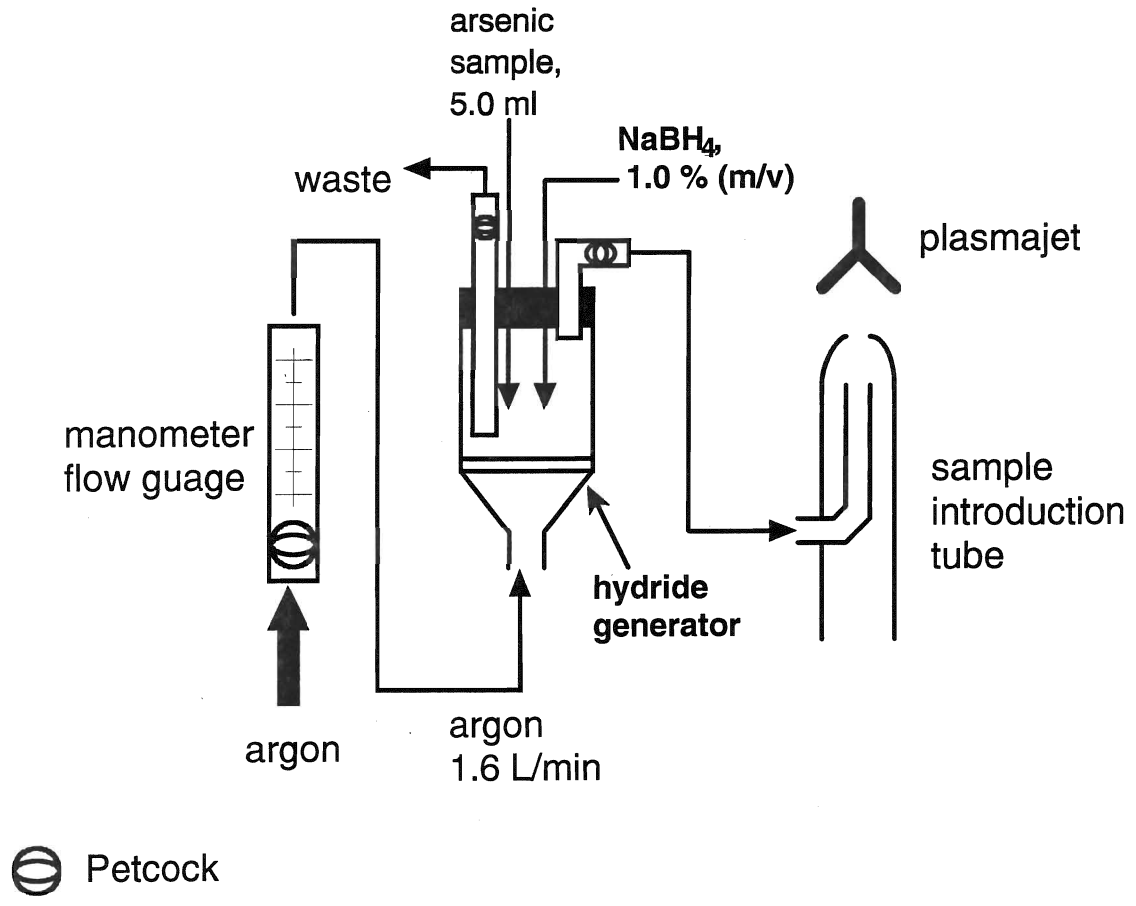
Instrumentation

The DCP-AES used for all studies was a SpectraSpan V equipped with a modified sample tube.[86] All results, before 12/93, were recorded on a Fisher Recordall series 5000 chart recorder. Hydride generation was done in a further modified Beckman hydride generator[86] as shown in Figure 3. The delay and drying columns plus the water U-trap were all removed. The arsenic containing sample was added to the hydride generator with a Brinkman adjustable volume Macro-Transferpettor. The volume of all samples was 5.00 mL. A disposable glass syringe was used to inject 1.0 mL of sodium tetrahydroborate(III). Argon gas flow through the hydride generator was controlled with a simple gas flow rotameter. All later work (after June 1993) used an in-house manufactured photon integrator for signal measurement. It is described in Appendix 1.

Reagents

The four arsenic species arsenite, arsenate, monomethylarsonic acid and dimethylarsinic acid were originally made up to standard stock solutions of 1000 ng mL^{-1} by

Figure 3. Schematics of batchwise hydride generation system.



the dissolution in 1 M nitric acid of arsenic trioxide, primary standard, (As_2O_3, AnalR, BDH, Toronto, ON), sodium arsenate ($Na_2HAsO_4 \bullet 7H_2O$, AnalR, BDH, Toronto, ON), monomethylarsonic acid, disodium salt ($Na_2CH_3AsO_3 \bullet 6H_2O$, Pfaltz and Bauer Inc., Stanford, CT, U.S.A.), sodium cacodylate ($(CH_3)_2AsO_2Na \bullet 3H_2O$, general reagent grade, BDH, Toronto, ON) respectively. Note that arsenic trioxide was initially brought into solution by dissolving in a minimum amount of 0.5 M NaOH and then filling to volume with 1 M nitric acid. The 1000 $ng \bullet mL^{-1}$ solutions were standardized against the primary standard As_2O_3 by normal nebulization in the DCP as described previously.[86] All calibration standard solutions of the arsenic species were made by the appropriate dilutions with 0.01 M analytical grade nitric acid (AnalaR, BDH, Toronto, ON; Mallinckrodt AR, Paris, KY, U.S.A.) in distilled water. In order to minimize any handling errors, the method of diluting was kept constant for the four different species by using equivalent denominations of pipetting and volumetric glassware. Tetrahydroborate(III) solution was 1.0% (m/V) $NaBH_4$ (98%, Sigma Chemical Co., St. Louis, MO, U.S.A.) in approximately 0.01 M sodium hydroxide and was made daily. The sodium hydroxide solution was made by the appropriate dilution of sodium hydroxide pellets (AnalR, BDH, Toronto, ON) . L-cysteine (Sigma Chemical Co., St. Louis, MO, U.S.A.) used for prereduction was added to the arsenic solutions as mass of L-cysteine per volume solution. Solutions were subsequently measured for pH. All argon gas used was welding grade. Glassware was cleaned regularly with fresh aqua regia. Comparison studies used potassium iodide (99.0%, BDH, Toronto, ON) and analytical grade hydrochloric acid (Mallinckrodt AR, Paris, KY, U.S.A.). All water used was distilled in glass.

DCP-AES Operating Settings

The samples were analyzed by the SpectraSpan V DCP-AES instrument using the operating parameters shown in Table IV. The photomultiplier voltage was adjusted so as to maintain the best signal-to-noise (S/N) ratio. Once set, the photomultiplier voltage was left constant during the all determinations. Sleeve pressure and nebulizer pressure were set by controls built into the DCP-AES whereas flow through the hydride generator was set externally as described below.

Batchwise Hydride Generation using L-cysteine

Prior to hydride generation, each arsenic sample contained 0.5% (m/V) L-cysteine. Each 50 mL sample was contained in a beaker and each beaker was heated in a boiling water bath for 180 s. The samples were then immediately cooled to room temperature in an ice bath. The samples were covered and left standing for at least one hour prior to DCP-AES measurement. The pH of the samples were accurately measured and brought to pH 2.25 with the dropwise addition of either 6 M nitric acid or 5 M NaOH. The production of the volatile arsines during hydride generation was accomplished by reacting 5.0 mL of arsenic sample with 1.0 mL of tetrahydroborate(III) solution. Hydride generation was performed with the aid of a Beckman hydride generator which was previously modified by Boampong *et al.*[86] Additional modifications included the complete removal of all drying and delay columns and the shortening of the ¼ in i.d. Nalgene transfer tube from the generator to the DCP-AES to

Table IV. DCP-AES operating parameters for batchwise hydride generation using both L-cysteine and potassium iodide reagents.

As wavelength	193.696 nm
slit size	
entrance	50 × 300 μm (w × h)
exit	100 × 300 μm (w × h)
sleeve pressure	50 psig
nebulizer pressure	disconnected
carrier gas flow	1.6 L min^{-1}

the shortest allowable length (approx. 600 mm). The arsine was stripped from solution and carried to the plasma of the DCP-AES by argon gas. The argon gas was continuously flowing through the hydride reaction chamber during the entire procedure. The argon flow through the vessel was regulated using a very simple adjustable valve manometer with a ball bearing flow indicator which was adjusted prior to the onset of sample measurement only and left untouched for the duration of all measurements. The flow was set with the petcock valves of the generator in the active hydride generation mode: flow to the waste drainage tubing was closed whereas the flow to the DCP-AES was open.

Batchwise Hydride Generation using Potassium Iodide as a Prereductant

Sample preparation for the comparison study first involved the appropriate dilutions of the arsenic species in hydrochloric acid at a specified concentration. Concentrations of HCl used were 1, 2, 4, 5, and 6 M. Potassium iodide was added to an aliquot from the final dilution of arsenic species in amounts ranging from 0-10% (m/V). The solutions were allowed to stand for at least 4 hours prior to hydride generation. The hydride generation procedure was analogous to the procedure used during hydride generation with the L-cysteine reagent. Hydride generation was performed by reacting 5.0 mL of the arsenic sample containing KI with 1.0 mL of tetrahydroborate(III) solution. The apparatus setup used here was identical to the setup described under Batchwise Hydride Generation using L-cysteine.

2. HPLC-FI-DCP-AES Arsenic Speciation Study

Instrumentation

The HPLC system consisted of a Waters model 510 gradient pump, a Waters model 501 pump, and a Waters Integrated Sample Processor (WISP) model 710B (Waters Assoc., Millford, MA, U.S.A.). Injections were initially made with the automated injector WISP 710B and subsequently with a Rheodyne model 7125 manual syringe-loading sample injector (Rheodyne Inc., Cotati, CA, U.S.A.) . Samples loops of 10 µl or 50 µl were attached to Rheodyne injector. All chromatograms illustrated in the discussion were obtained when using the manual Rheodyne injector.

A guard column (Hamilton Company, Reno, NV, U.S.A.) was used with every run. The ion exchange column was a 100 × 4.1 mm PRP-X100 (Hamilton Company, Reno, NV, U.S.A.) strong anion exchange column. The strong anion exchange resin is a spherical 10 µm cross-linked styrenedivinylbenzene copolymer with attached trimethylammonium functional groups.

The DCP-AES instrument was the same as above except for the following modifications. Signals from the DCP-AES were no longer processed by its internal electronics but were generated by a new photon integrator built by the Electronics Shop, Brock University (see Appendix 1. for details of construction). Analog signals generated by

the photon integrator were digitized by an Analog/Digital Converter (A/D) also built by the Electronics Shop, Brock University. Signals were collected by a 386SX 25 MHz with math coprocessor PC using a data acquisition program designed at Brock University by Tom McDonald. Post-processing of the signals was performed using the commercially available computer program Spectra Calc, Galactic Industries Corporation.

Flow injection reagents were pumped using a Minipuls 3 peristaltic pump (Gilson: Mandel Scientific Co. Ltd., Rockwood, ON) and SMA Flow Rated Pump Tubes (Technicon Instruments Corp., Tarrytown, NY, U.S.A.). The drain tubing was 3/16" Tygon tubing (Tygon Corp.: Fischer, Unionville, ON).

A continuous hydride generator was designed in-house for the analysis. The glassware was manufactured in-house also. Details of the design is shown in Figure 4. A tightly fitting rubber stopper was fixed into the top of the glass cylinder. The rubber stopper contained two holes (i) and (ii):

(i) A glass tube with o.d. 4 mm and i.d. 2 mm was fitted into one hole. This glass tube was used for the introduction of the arsenic species and all reagents into the hydride generator. An SMA Flow rated pump tube was fitted over the glass tube for connection between the stainless steel lines and the HG chamber.

(ii) A glass tube with o.d. 6 mm was fitted into the second hole. Approximately 200 mm of 3/8' (10 mm) tygon tubing was used to transfer all gaseous species from the hydride generator to the DCP-AES. One end of the tubing was fitted over the glass

tube on the generator and the other end was fitted onto a glass petcock just prior to the glass plasmajet introduction tube. The glass petcock was used to stage down the transport tubing and as an adapter in order to join two different size tubings. The glass tube on the hydride generator side of the petcock had an o.d. of 6 mm and the DCP-side glass tube had an o.d. of 4 mm. There was then 200 mm of i.d. 4 mm SMA tubing that completed the attachment to the sample introduction tube below the plasmajet shown in Figure 3.

The waste was pumped out of the generator through the tygon drain tubing which fit tightly into the glass waste tube shown in Figure 4. The argon carrier gas was fed into the chamber via tygon tubing which was fit tightly over the argon inlet tube. The argon flow was controlled with a very simple petcock manometer with a ball bearing flow indicator. The name of the manometer manufacturer was not evident on the outside of the instrument.

Many variations of the FI system shown in Figure 5 were tested. Most of the variations tested were attempts towards an effective mixing and reacting scheme. The final scheme chosen had the sodium tetrahydroborate (III) line and the arsenic/cysteine line joined approximately 10 cm before the chamber. The present author believes this scheme had demonstrated the best HG efficiency. Other less successful attempts involved the introduction of two separate lines carrying respective sodium tetrahydroborate (III) and arsenic/cysteine solutions into the chamber. Various introduction geometries of the two separate lines were tested in order to increase HG efficiency. Ineffective mixing appeared

Figure 4. Schematic representation of the continuous hydride generator.

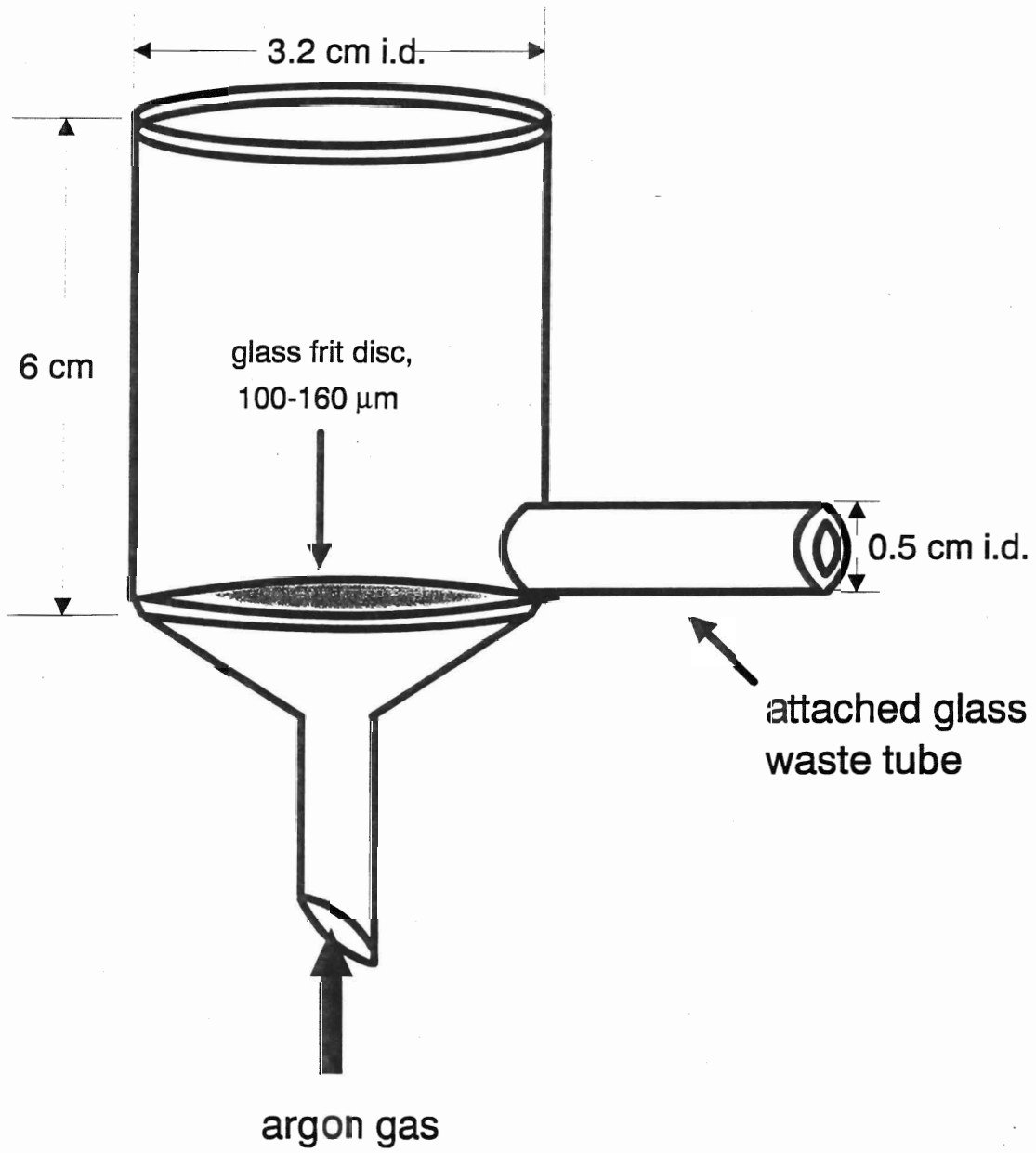
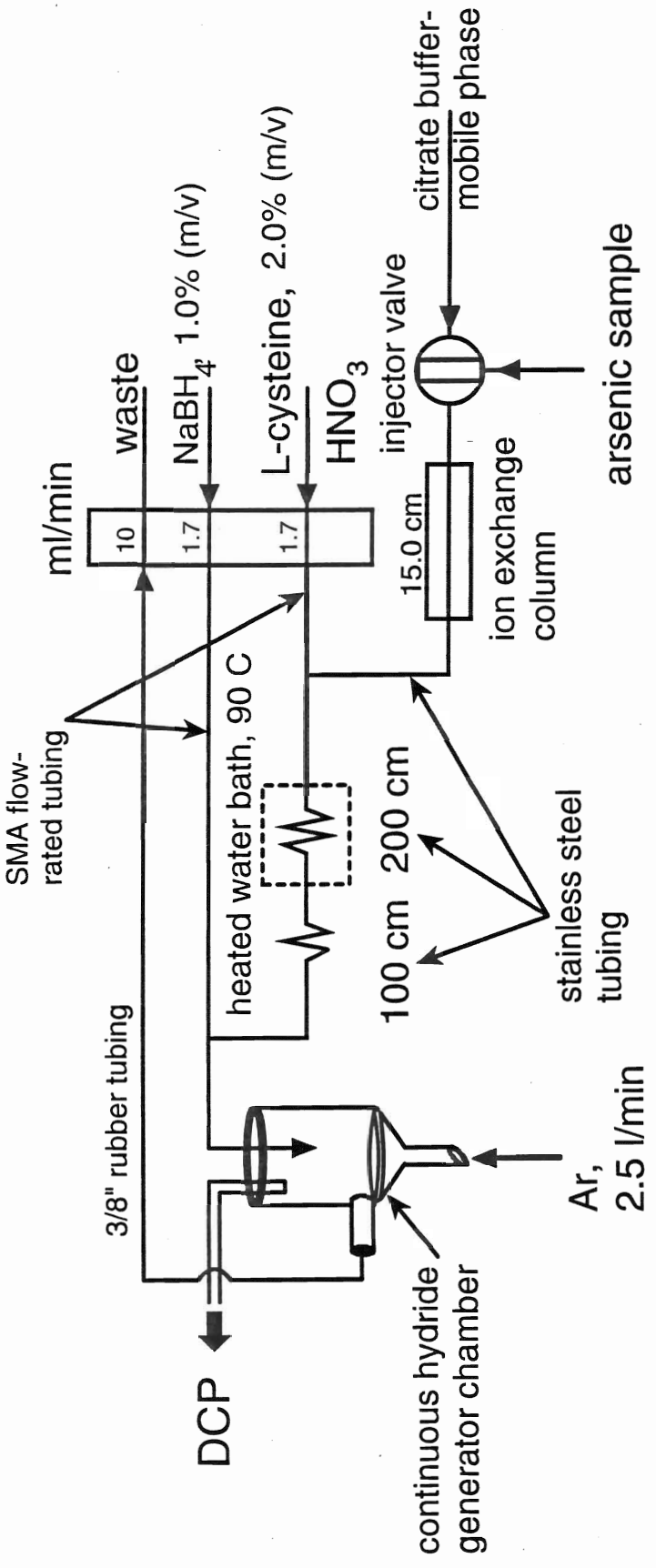


Figure 5. HPLC-FI-DCP-AES schematic diagram used for arsenic speciation.



to be the problem of the less successful attempts. Results were not recorded for the less successful attempts.

Instrumentation Operating Parameters

The operating parameters for the FI- DCP-AES setup are described collectively in Table V and Figure 5. The photomultiplier voltage of the DCP-AES was adjusted so as to maintain the best signal-to-noise (S/N) ratio. Once set, the photomultiplier voltage was left constant during the all determinations. Sleeve pressure and nebulizer pressure were set by controls built into the DCP-AES whereas flow through the hydride generator was set externally as described previously in the Batchwise Hydride Generation section.

Table VI describes the elution program used for the arsenic speciation determination. Equilibration time between the onset of subsequent runs was 7 min. During the equilibration time the mobile phase was switched back to the composition at t=0 of the run.

Table V. Operating parameters for HPLC-FI arsenic speciation.

Parameter	Setting
As wavelength	193.696 nm
slit size	
entrance	50 × 300 μm (w × h)
exit	100 × 300 μm (w × h)
sleeve pressure	50 psig
nebulizer pressure	disconnected
Argon gas flow through hydride generator	2.5 l min^{-1}
sodium tetrahydroborate (III)	1.0% (m/v) 1.7 mL min^{-1}
nitric acid	0.25 M
L-cysteine	2.0% (m/v) 1.7 mL min^{-1}

Table VI. Gradient elution program for arsenic speciation by IC.

time (min)	% eluent A	% eluent B	flowrate ($mL \bullet min^{-1}$)
0	100	0	1.0
3.5	0	100	1.0
5.0	0	100	1.5
12.0	100	0	1.0

eluent A = 0.0001 M citrate / 5% MeOH, pH = 9.0

eluent B = 0.0005 M citrate / 5% MeOH pH = 9.0

Reagents

All arsenic sample preparation used the same procedure and reagents described at the beginning of the Methods section 1.

Analytical reagent grade methanol (Omnisolve, BDH, Toronto, ON) used was to prepare the 5 % (V/V) methanol mobile phase. Sodium citrate, as the trisodium salt, was 99.0% pure (BDH, Toronto, ON).

All eluents were filtered prior to use with 0.45 μm cellulosic filters (Micron Separations Inc., Westboro, MA, U.S.A.) in order to increase the column lifetime. All eluents were degassed either by filtering under a water aspirated vacuum in an ultrasonic bath for aqueous solutions or with pure helium for organic solvents.

Preparation of Buffered Mobile Phase

The speciation method used two citrate buffered solutions of 0.0001 M and 0.0005 M. Each eluent contained 5% methanol unless otherwise indicated. The pH was adjusted to approximately 9.0 with the dropwise addition of 1.0 M sodium hydroxide. According to the certificate of analysis provided by BDH, a 5 % solution of tri-sodium citrate has a pH of 8.2 to 9.2.

RESULTS AND DISCUSSION

1. Preliminary Study of Arsenic Speciation using L-Cysteine

As stated in the introduction, the inorganic trivalent arsenical has better recoveries than the pentavalent arsenical during hydride generation under the conventional acidic HG conditions primarily because the pentavalent species must first be reduced to As(III) before arsine (AsH_3) can be produced. Therefore it would be beneficial to prereduce any As(V) to the more sensitive As(III) prior to the onset of a total inorganic arsenic determination. Previously in this laboratory it was demonstrated that L-cysteine at a concentration of 0.5 % (m/V) in 0.01 M nitric acid was an effective prereducing agent for As(V). L-cysteine also enhanced signal generation at relatively low acidic conditions and reduced interferences during HG for both As(III) and As(V).[56] Therefore, an efficient total inorganic arsenic (As(III) + As(V)) determination is possible when using L-cysteine. Continuing on with the development of an effective and suitable arsenic speciation analysis using HG would bring attention to the methylated arsenic species MMAA and DMAA which are next two most naturally abundant species. The arsenic in both of these methylated species exists in the pentavalent oxidation state and therefore these species would require a prereduction step before HG determination. Therefore the study into the effectiveness of L-cysteine to prereduce the methylated species and thus enhance their sensitivity during HG will now be the focus of this discussion.

A practical starting point for this work was to repeat the conditions for the batchwise hydride generation of the inorganic arsenicals but the analyte would now be the organic arsenicals. The HG generation medium was L-cysteine at 0.5% (m/V) in 0.01 M nitric acid. Signals produced by the organic arsenicals were measured and compared to the signals produced by As(III), which was considered the standard here. Present studies demonstrate that the prereduction of the two inorganic arsenicals was fast at room temperature prior to HG. For example, the inorganic analyte required no more than 20 min in the HG medium in order to produce complete recovery. Similarly, prereduction at room temperature of DMAA was fast, but prereduction of MMAA was slow, as shown in Figure 6. We believe that the poor recovery of the MMAA was a kinetic problem because the recovery did increase with time, suggesting that both the reaction with L-cysteine was incomplete as indicated in Figure 8 by the comparison of peak heights between heated and room temperature MMAA. In addition, similar arguments which substantiate our suggestions were presented from other authors whom observed the same problem of relatively slow arsine generation when using a concentrated acid technique. Therefore the MMAA sample required heating in order to assure complete reduction by L-cysteine. Figure 7 shows the recovery effect of heating on MMAA. These solutions were 50 mL and were heated in 100 mL flasks. The solution samples in these containers had a very low surface area to volume ratio and therefore it is safe to assume that time required for complete reduction by L-cysteine will be greatly decreased by a more efficient heat transfer process. In fact, this was proven during work with HPLC-FI-DCP and will be discussed later. In later experiments, all species were heated in order to assure complete reaction with the L-cysteine and to maintain consistency for the

Figure 6. Arsenic recoveries, while standing at room temperature, of 50 ng•mL^{-1} MMAA and DMAA in a solution of 0.5% L-cysteine (m/V) and 0.01 M nitric acid at pH 2.25.

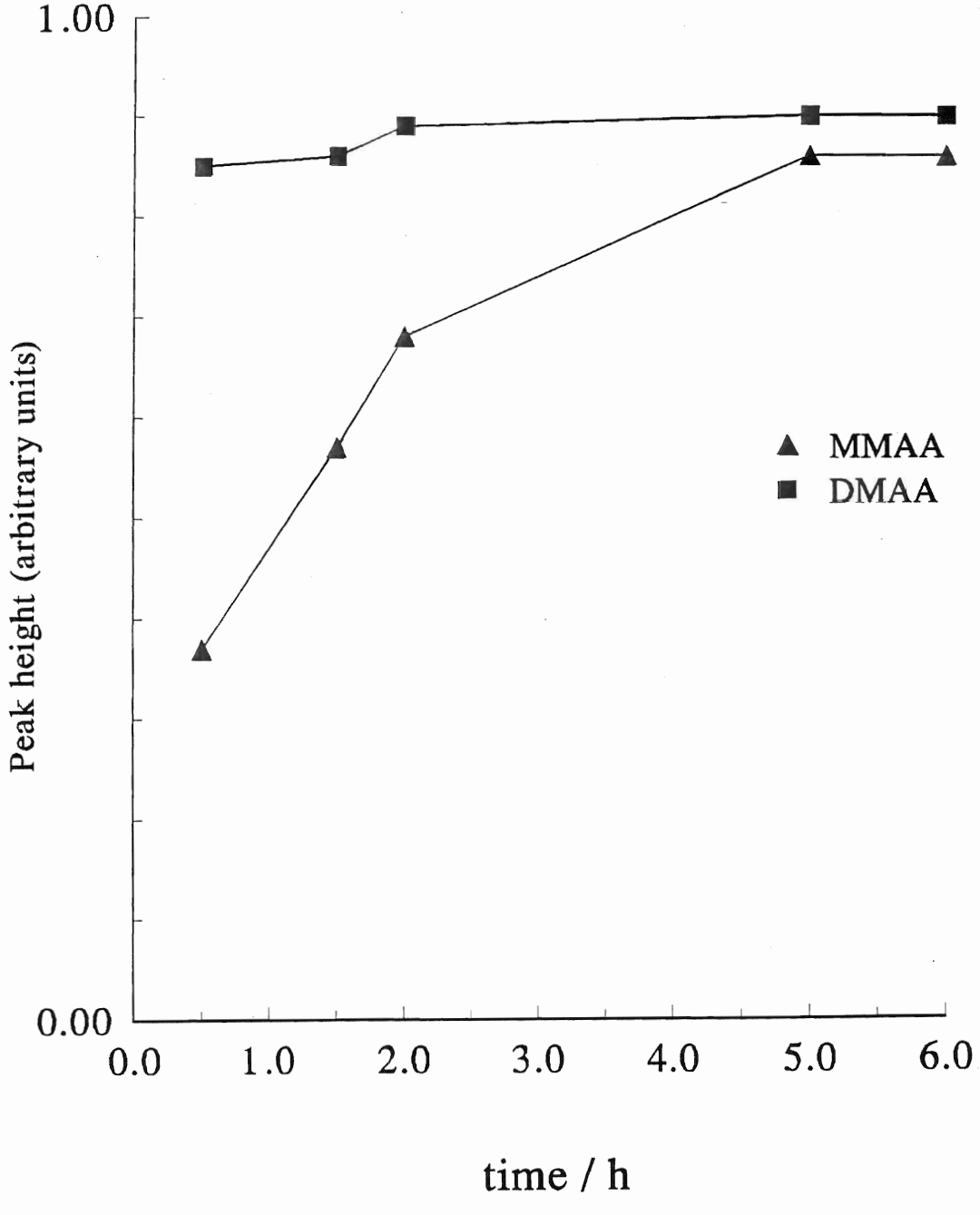


Figure 7. Effect of heating in a boiling water bath on the recovery of 50 ng mL^{-1} MMAA using 0.5% (m/V) L-cysteine reagent. All analyte peak heights were normalized against 50 ng mL^{-1} As(III).

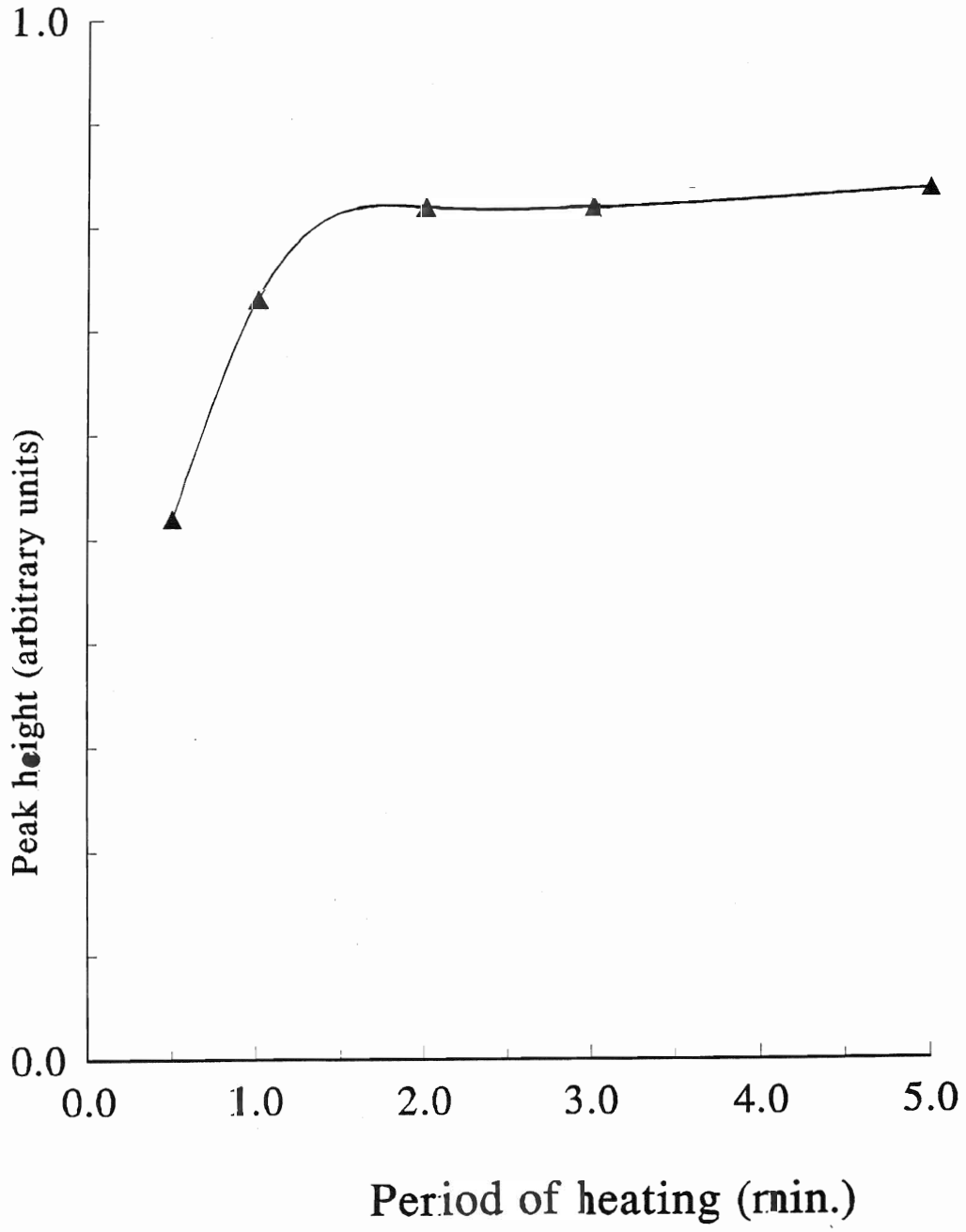
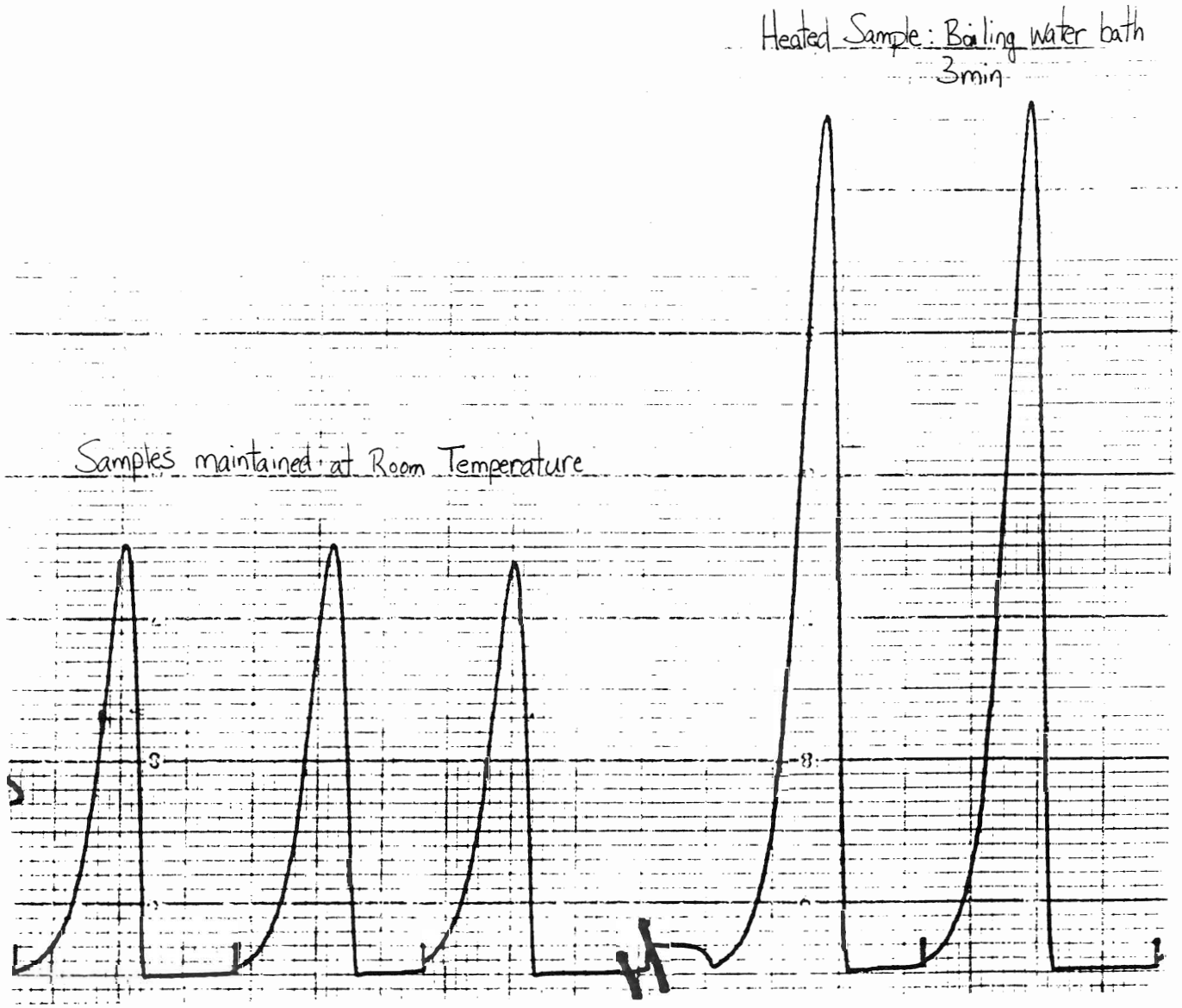


Figure 8. Hydride generation peaks of 100 ng•mL^{-1} MMAA comparing heated and room temperature samples for the degree of prereduction by L-cysteine. HG medium was 0.5 % (m/V) in 0.01 M nitric acid at pH 2.25.



sample preparation of all species. No detrimental effects were observed by heating all the samples.

Effect of pH

Hydride generation using L-cysteine was reported by Chen *et al.*[56] to be pH dependent. Chen *et al.* used a solution with pH 2.10-2.15 to obtain maximum recovery of As(V) and As(III). During the present investigation, the determinations of MMAA and DMAA were, similarly, observed to be pH dependent. Therefore a study of the effect of pH on DMAA and MMAA determinations were pursued. In Figure 9, the slope of the sensitivity plot from DMAA is more stable than the slope of the plot form MMAA, which is shown in Figure 10, and therefore DMAA exhibits a lesser degree of sensitivity to pH change than does MMAA. But even though MMAA was more sensitive to pH adjustment, it was observed that both of these species give maximum recovery at pH 2.25. Therefore a single medium consisting of L-cysteine reagent in a 0.01 M nitric acid at pH 2.25 allows for maximum recoveries from all 4 species.

Recoveries, Precision and Detection Limits

After the conditions that allowed maximum recovery during HG were determined, as described in the methods section, the detection limits and precision for each species was measured. Precision was measured as the standard deviation of 10 consecutive peak heights

Figure 9. Effect of pH on the recovery of 50 ng•mL^{-1} DMAA sample using 0.5% (m/V) L-cysteine reagent. Peak height was normalized against 50 ng•mL^{-1} As(III) determined concurrently.

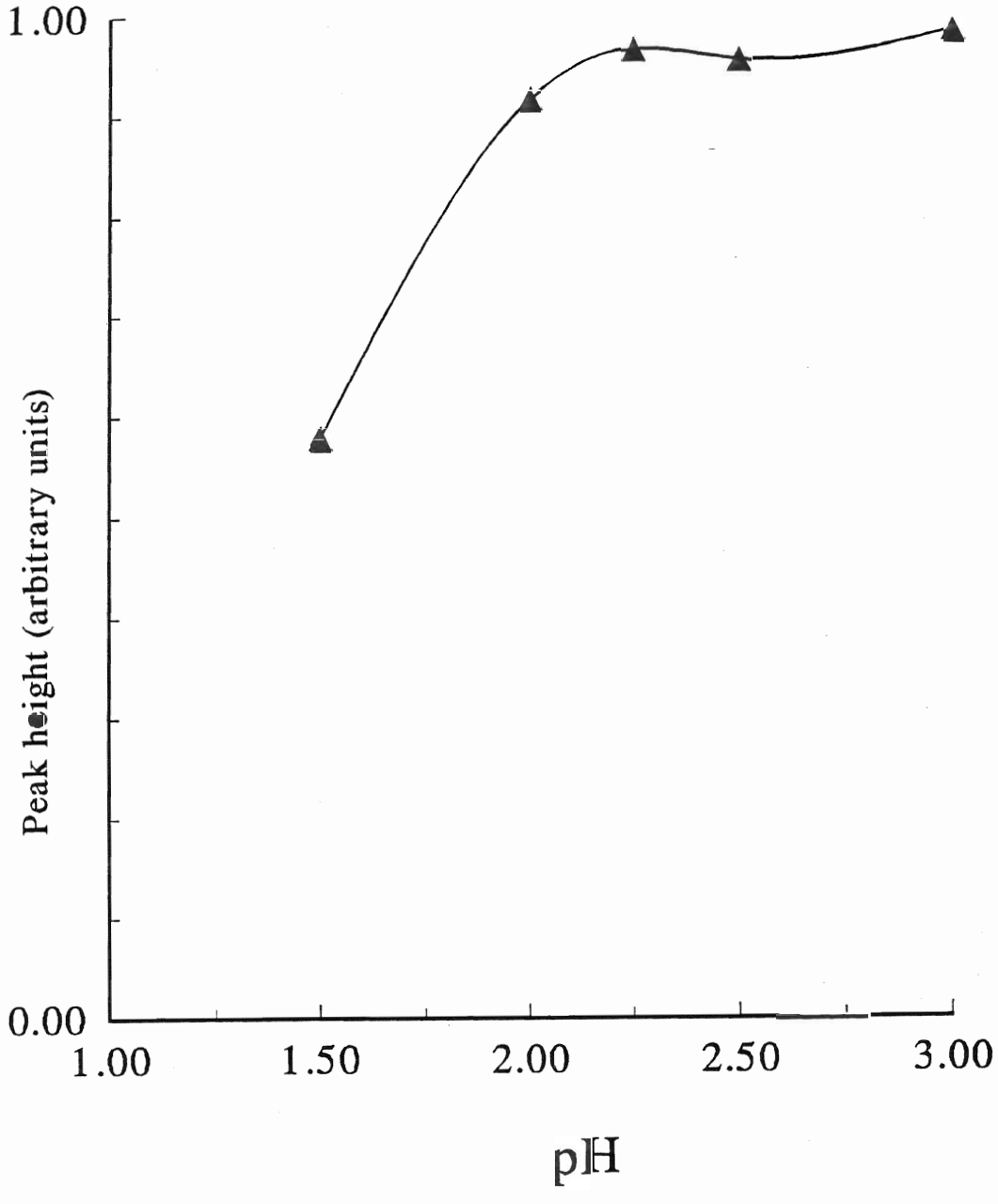
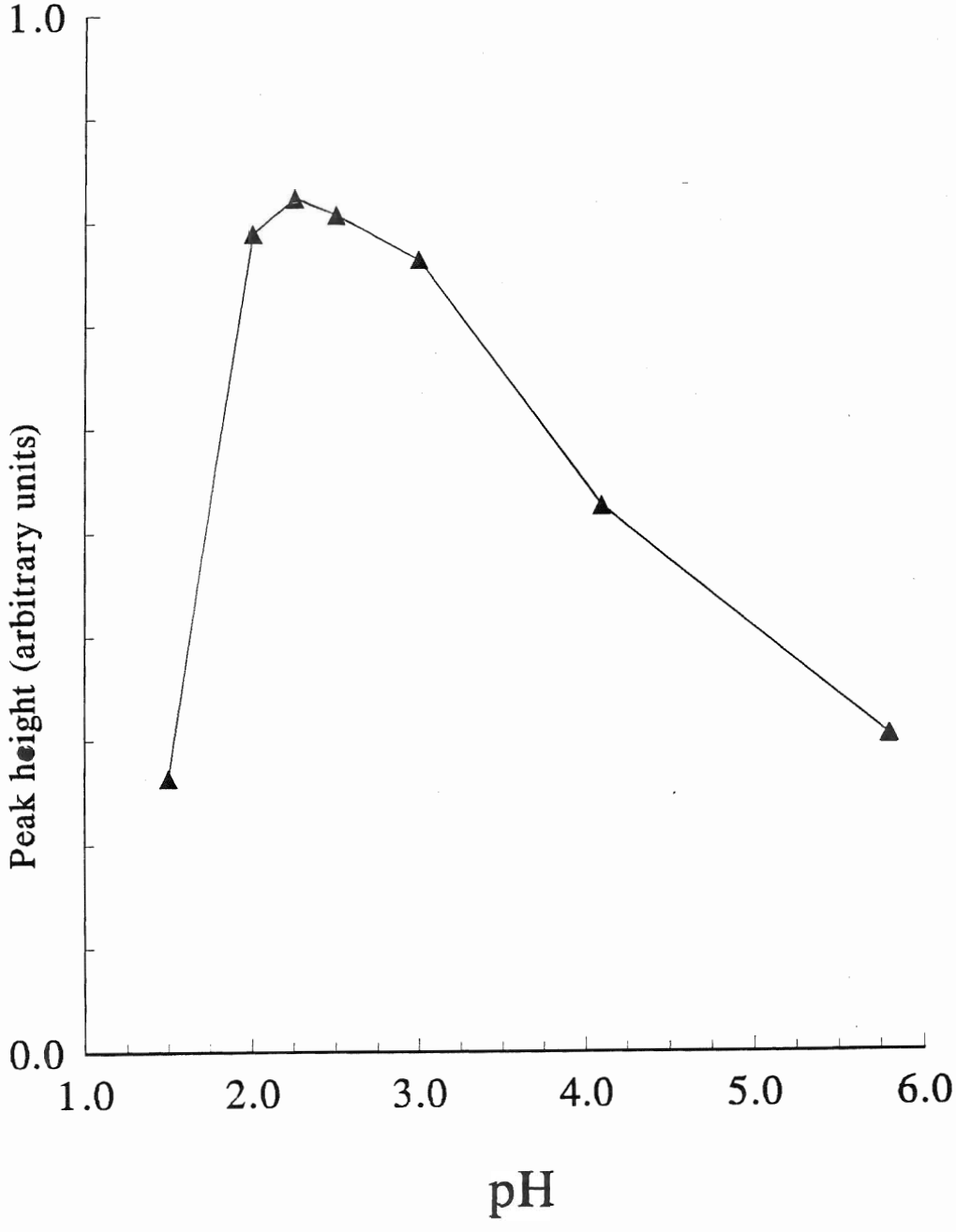


Figure 10. Effect of pH on the recovery of 50 ng•mL^{-1} MMAA sample using the L-cysteine reagent. Peak height was normalized against 50 ng•mL^{-1} As(III) determined concurrently.



at 50 ng•mL^{-1}. During the course of experiments, the standard deviation was never worse than 3.0%. Precision values are shown in Table VII.

Detection limits were calculated from calibrated standards of 100, 50, 20, 10, 5, 1 ng•mL^{-1}, (plus 0.5 ng•mL^{-1} for As(III) only), and a blank solution. The detection limit was defined to be three times the standard deviation of the predicted intercept as defined in Miller and Miller.[87] The correlation coefficient for linear regression lines, for the range of 1-100 ng•mL^{-1}, of all four species were no less than 0.9950, and thus indicated excellent fit of the data. Detection limits are shown in Table VII. The absolute detection limit for the 5.0 mL analyte sample ranged from 4.0 - 6.0 ng of arsenic for the respective species.

Recoveries of the four species were calculated from the precision values experiment of 10 consecutive arsenic signals at 50 ng•mL^{-1} as mentioned above. Complete arsenic recovery for As(III), As(V), and DMAA was possible while MMAA had a 86% recovery. These values were calculated by comparing with As(III) because Chen *et al.*[56] stated that As(III) exhibited complete arsine recovery from the solution matrix.

Table VII. Precision values and detection limits for the determination of the four arsenic analytes by hydride generation using 0.5 % (m/V) L-cysteine in 0.01 M nitric acid at pH 2.25.

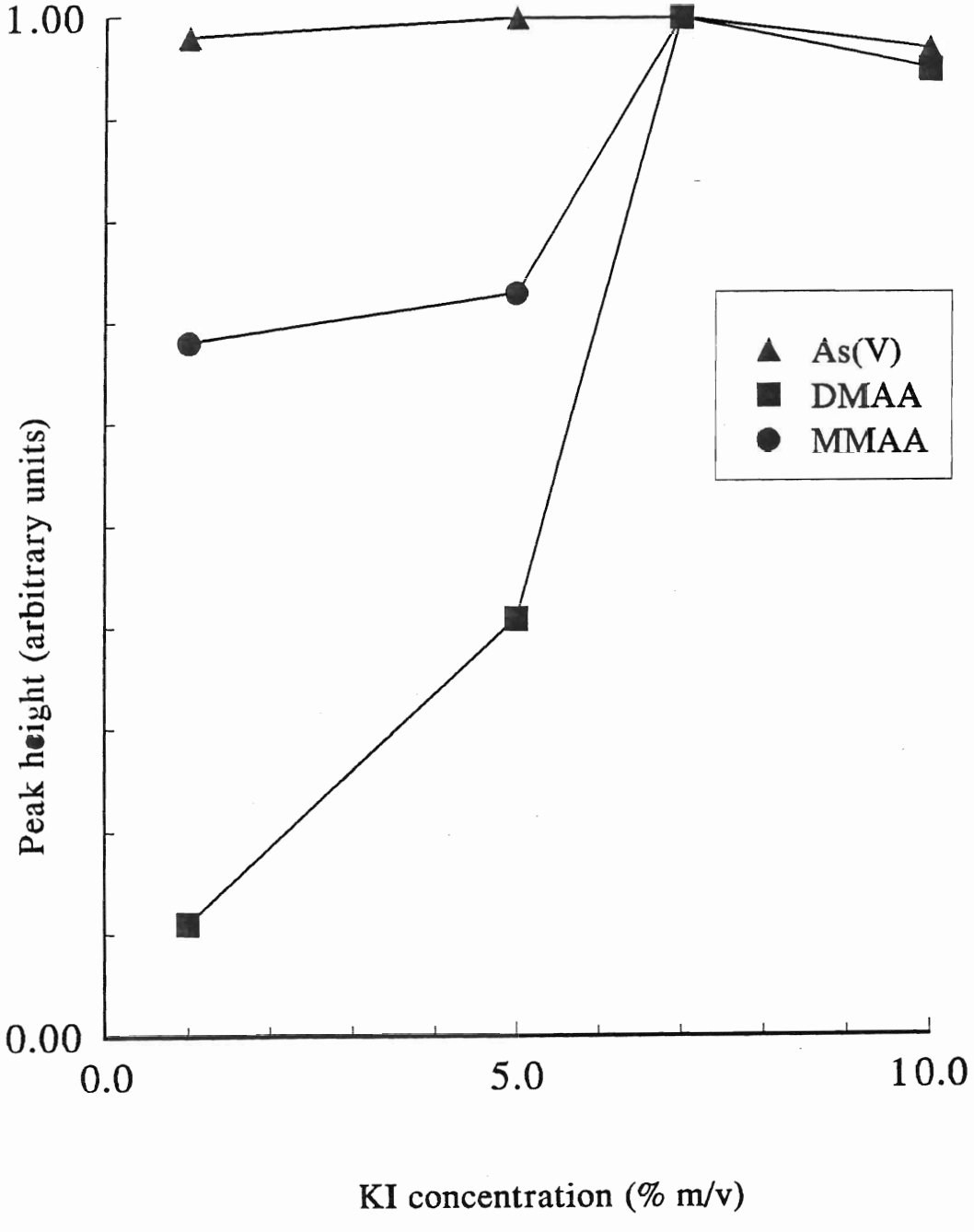
Arsenic Species*	Precision (%)	Detection Limit (ng mL^{-1})	r-squared values
As(III)	1.8	1.2	0.9961
As(V)	2.5	0.8	0.9950
MMAA	2.6	1.1	0.9988
DMAA	2.6	1.0	0.9982

* The concentrations of the standards for calibration were 100, 50, 20, 10, 5, 1 ng•mL^{-1}, (plus 0.5 ng•mL^{-1} for As(III) only), and a blank.

Comparison with Iodide Method

The most accepted reagent for prereduction of arsenic prior to HG is potassium iodide (KI). Therefore it was appropriate to compare the prereducing capabilities of L-cysteine with iodide in order to prove the validity of the L-cysteine reagent. A quick preliminary study was performed on the conventional hydride generation medium by adjusting the concentrations of both HCl and KI in order to maximize signals. The procedure followed is outlined in the method. The arsenic in iodide solutions were left standing for 4 hours prior to hydride generation because this was the time required for complete prereduction of As(V) to As(III) as demonstrated by Haring *et al.*[43] Furthermore, iodide is only effective in concentrated acid solutions and, as such, was used in HCl concentrations between 1 and 6 M. This range of HCl concentrations did not vary the sensitivity significantly, though it was observed that 5 M HCl produced slightly better sensitivity. Therefore a 5 M HCl solution was chosen for direct comparison with the cysteine reagent. The literature reports differing KI concentrations for studies involving either batch or continuous FI determinations. Therefore the prereducing effect of KI on the pentavalent species during HG was studied and shown in Figure 11. Experiments were carried out with iodide concentrations ranging from 0-10% (m/v). The data plotted in Figure 11 was the ratio of signal heights from the hydride generation of each particular arsenic species obtained when using the KI reagent compared immediately to an analogous procedure using the 0.5% (m/V) L-cysteine reagent. The maximum recovery for the KI reagent with regards to all arsenicals was determined to use

Figure 11. Effect of potassium iodide concentration on the recovery of 50 ng•mL^{-1} of the three species: As(V), MMAA, and DMAA. Peak heights were normalized against the respective arsenic species in 0.5% (m/V) L-cysteine reagent



7% KI in 5 M HCl. We observed that the methylated species were more sensitive to changes in the iodide concentration than were the inorganic species.

It was observed that the iodide reagent, while in the hydride generation chamber, produced excessive foaming compared to the cysteine reagent. The excess foaming was a result of the high iodide salt concentration. Foaming did not seem to affect the sensitivity of the arsine species in this experiment, though could be a potential disadvantage when used in conjunction with other matrices that also foam. We observed that the sensitivity obtained when using iodide was always less than when using cysteine as shown in Figure 12. In fact, it was much inferior with regards to the methylated analytes, as can be seen in Figure 12. The recoveries obtained by using iodide for MMAA and DMAA determination were 79% and 22%, compared to the respective species prereduced with L-cysteine. The especially poor recovery of DMAA when using the iodide regent was accompanied with a very high relative standard deviation of 22 %. The results comparing recoveries between the two reagent for all species normalized to the respective arsenical are shown in Table VIII.

The peak shapes, produced when using L-cysteine, were identical for the four species whereas the shapes differed when using iodide. The results are shown in Figure 13. L-cysteine-treated samples reacted rapidly with borohydride exhibiting tall sharp peaks whereas dimethylarsine production using the iodide reagent was slow and exhibited broader peaks

Figure 12. HG comparison between L-cysteine and potassium iodide reagents for the determination of four arsenic species. All peak heights were normalized against the peak obtained from As(III) in 0.5% (m/V) L-cysteine.

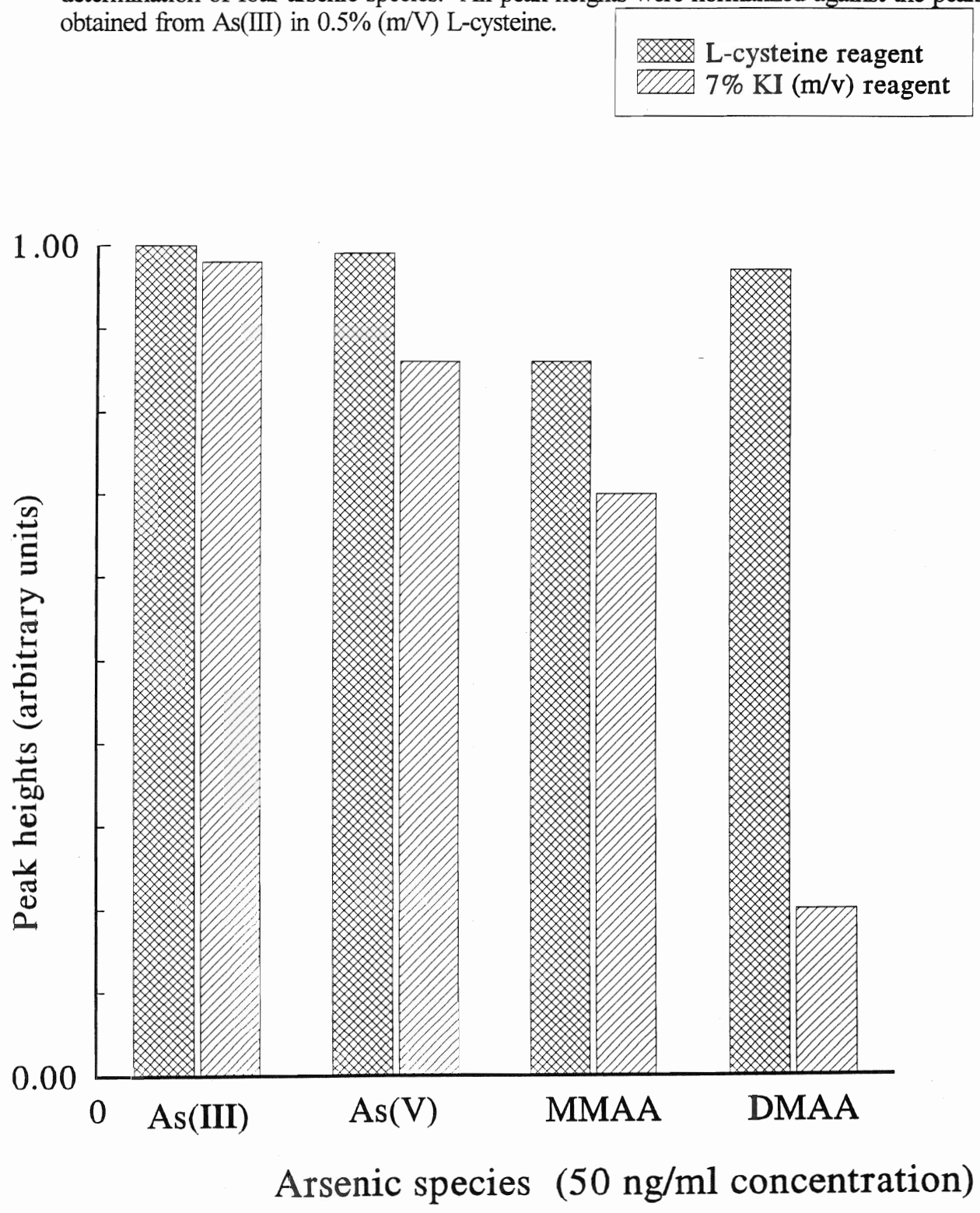
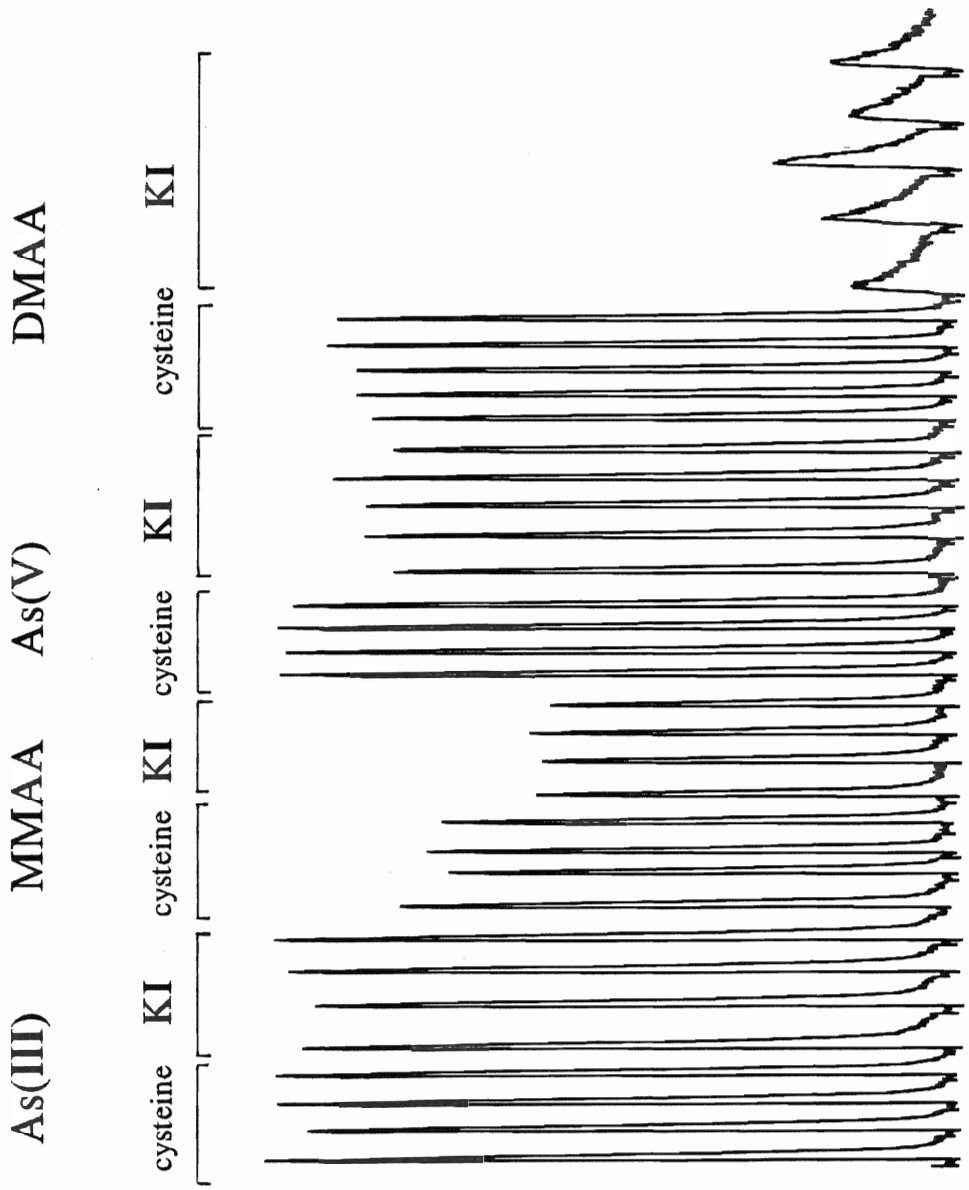


Table VIII. Comparison of HG recoveries between each arsenic species obtained from using either L-cysteine or potassium iodide prereducing agents.

arsenic species	prereducing agent recovery, r.s.d.	
	L-cysteine	potassium iodide
As(III)	1.00, 2.7%	0.98, 2.6%
As(V)	1.00, 0.6%	1.00, 0.87%
MMAA	1.00, 3.7%	0.79, 1.9%
DMAA	1.00, 3.2%	0.22, 22%

Recoveries were calculated from Figure 11. All concentrations were 50 ng•mL^{-1}. Recoveries in iodide were normalized against the respective arsenical in 0.5% (m/V) L-cysteine reagent.

Figure 13 Typical peak shapes of hydride generated signals from the four arsenic species. Concentrations of all species was 50 ng•mL^{-1} except the MMAA concentration was 40 ng•mL^{-1}.



with significant tailing. This observation, using concentrated HCl, is in agreement with other studies in which the hydride was collected for an extended duration prior to introduction into the spectrometer because of its relatively slow production.[49]

In conclusion, we have demonstrated that L-cysteine can enhance hydride generation of the organic arsenicals with the same effectiveness as the inorganic arsenicals. In fact, very similar sensitivities were produced using identical conditions for all four species during batchwise hydride generation. We suggest that L-cysteine reacts with the pentavalent arsenic in the methylated species to form either a coordinated sulphur ligand compound or the respective arsenous acid species. This suggestion is based on the following arguments:

(a) Cysteine was previously shown to reduce inorganic arsenate to arsenite and thereby allowing hydride generation of arsenate sample in a low acid concentration medium.[56]

(b) Anderson *et al.*[55] observed that a mercaptoacetic acid reaction medium for hydride generation produced similar sensitivities for all four arsenic species. He suggested that a "sulphur containing ligand" was important in the hydride reaction.

(c) Ebdon *et al.*[51] demonstrated that inorganic arsenic coordinated with methylthioglycolate (MTG) and formed the stable volatile $As(MTG)_3$ species. In addition, the authors cited another study which demonstrated that MMAA and DMAA formed stable volatile compounds with MTG (e.g. (dimethylarsino)MTG and (monomethylarsino)MTG_2). The arsenic in each methylated species was reduced during the coordination to form the methylthioarsinite and a disulphide compound.

(d) Cullen *et al.*[52,53] prepared and identified S-(dimethylarsino)cysteine and S-(methylarsino)dicysteine from dimethylarsinic acid and monomethylarsonic acid, respectively, with cysteine in a neutral pH solution. Similar products were identified with trimethylarsine oxides.

In addition, the sulphydryl group of the cysteine was previously shown to react with the sodium tetrahydroborate(III) to form a thio(trihydroborate(III)) coordinated compound.[30]

The application of this new found arsenic-signal enhancing HG medium containing L-cysteine towards a continuous FI hydride generation determination of these species, after separation by LC, can now be investigated with the promise of similar sensitivities between the species. The L-cysteine reagent would also be advantageous towards an FI procedure because the low acid concentrations required are much less harsh than the concentrated acid conditions necessary using conventional techniques and therefore should reduce operating costs and would be favourable to both operator and equipment.

2. Arsenic Speciation

The method chosen for the chromatography of the arsenic species was ion exchange chromatography (IC) using a Hamilton PRP-X100 column. Anion exchange chromatography is a logical choice to separate these four species because they all exist as anions in aqueous solutions. The parameters under consideration during the development of this IC method

included the ion-exchanger type; ion type, pH, and ionic strength of mobile phase; flow rate, organic modifier; and temperature.

First of all, the reason for choosing IC over reverse-phase ion-pairing chromatography (IP), which is another option for arsenic speciation, was based upon the following facts. Mobile phases in IC are relatively simple because they are most often single phase and, at most, binary phase, whereas IP are more complicated to develop because the mobile phases are often ternary. Another factor which can complicate the develop of IP is the choice of an ion-pairing agent which is not necessary during IC. Therefore, IC was chosen as the method to pursue because there are less variables to account for during the development of a procedure.

The reason for choosing a Hamilton PRP-X100 column was based upon the facts it was a strong anion exchanger which is best suited for separating weak acids and the resin contains a polymer substrate. A strong anion exchanger was chosen because of the effect that pH has upon the exchange capacity of the resin. Strong anion exchangers can be used with solutions at pH below 10, while maintaining maximum exchange capacity, whereas maximum exchange capacity of weak anion exchangers is observed for solutions with pH below 6, only.[88] Therefore a strong anion exchanger was selected because it can be used with maximum efficiency at the pH desired for this work, which will be discussed next. This column with a polymer 'backbone' was favoured over other silica 'backbone' columns because polymer resin is stable for a pH range from 1-13 whereas silica must be kept below

pH 8 for prolonged use. Although other limitations exist for polymer substrates, such as an increased degree of swelling over silica substrates, the reason for favouring a polymer substrate because of pH stability will be made more evident in the following paragraphs.

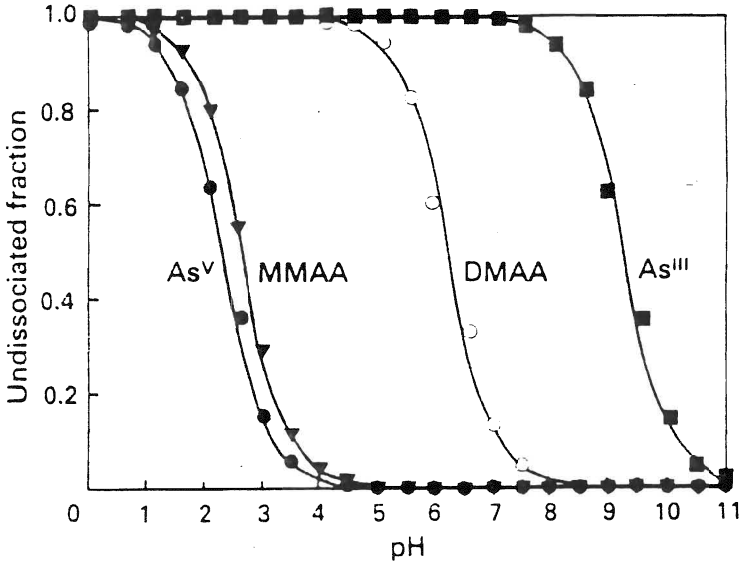
In IC, an ion type or salt must be chosen to begin any ion-exchange work. The salt is chosen based upon its ionic strength and affinity for the stationary phase because its interaction with ionic sites on the stationary phase and its competition with the analyte for these sites will aid in the resolving of the analytes into individual species. In addition, depending upon the problem at hand, the salt can either have buffering properties or no buffering properties.[89] The decision made about the choice of a salt was based on the following arguments. The four species have differing pK_a values, shown in Table IX, and the degree of ionization of the arsenical species is dependent upon the acidity of the matrix which is graphically shown in Figure 14. These dissociation values were calculated from the pK_{a1} values. Bearing in mind that only ionized substances interact with ion exchange resin and from the facts presented in Figure 14, it was decided that a basic pH buffer salt should be used to maintain a high degree of ionization for the arsenicals. A citrate buffer was chosen as the mobile phase because it could maintain a basic pH. For instance, a 5 % solution of tri-sodium citrate in distilled water will have a pH of 8.6 to 9.2 as described by the certificate of analysis provided by BDH chemicals.

Table IX. Molecular form and acid dissociation constants for the inorganic and methylated arsenicals.

species	acid form[67]	pK_a[91]
As (III)	$AsO(OH)$	9.3
As (V)*	$AsO(OH)_3$	2.3 6.9 11.4
MMAA*	$CH_3AsO(OH)_2$	2.6 8.2
DMAA	$(CH_3)_2AsO(OH)$	6.2

* these species are polyprotic and as such have more than one pK_a value as shown corresponding to each dissociation.

Figure 14. Relationship between pH and degree of dissociation of the four main arsenic species. This graph was reproduced from ref. 54.

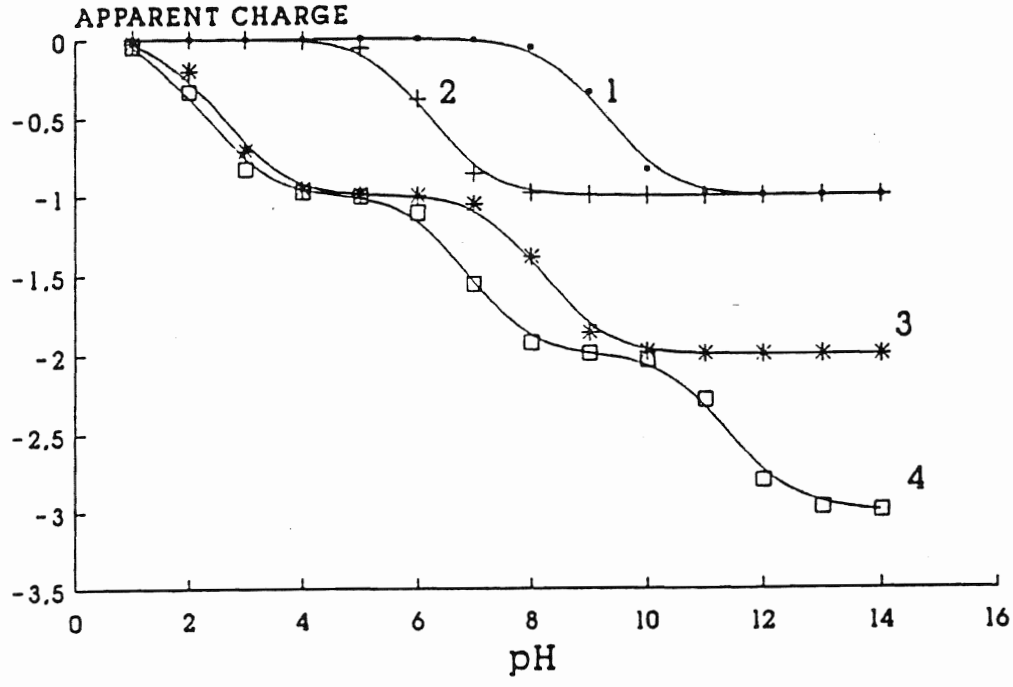


As described elsewhere, the order of elution by IC was dependent on the charge of the analyte and the pH of the eluent. Spall *et al.*[90] state that As (III) and DMAA, which are singly charged, eluted before MMAA and As (V) which are doubly charged. In fact, it is more accurate to assume the following at the given mobile phase pH of 8.92. As(III) eluted close to the solvent front because it was not dissociated to any significant extent and was not held by the stationary phase. DMAA, which has a single proton, was fully dissociated at this pH and had a charge of -½ since the single charge was delocalized over two oxygen bonds. Therefore DMAA eluted second, after As(III), but before MMAA which had a charge of approximately -0.66. As(V), with an approximate charge of -0.75, eluted last because it had the highest charge and therefore had the strongest interactions with the stationary phase. The charge on both MMAA and As(V) was approximated by following the same arguments as the charge calculation for DMAA and considering the acid dissociation constants for each species. In a recently published study, a similar but more descriptive reason for the elution order was explained. These authors stated that the eluting characteristics of the analytes were the result of their apparent charge which was dependent upon the acidity of the solution.[91] The apparent charges, which were accurately determined in the above mentioned study, are shown in Figure 15. The apparent charges between each species in order of increasing charge were:

$$As(III) < DMAA < MMAA < As(V)$$

which follows the same trend as described previously. This graphical representation strengthens the argument for using a slightly basic buffer in the pH range 7-9 for IC in order

Figure 15. Relationship between apparent charge and pH for the four arsenic species. This graph was reproduced from ref. 91.



1 = As(III)

2 = DMAA

3 = MMAA

4 = As(V)

to obtain the best possible separation since the charge plots of the data were most separated in this pH region.

In this study, the elution was carried out in a pH of approximately 9.0 and the behaviour observed was identical to the studies mentioned above. As (III), which is very slightly charged eluted first, DMAA, which was singly charged, eluted second, MMAA, with a charge approaching -2, eluted third, and As (V), which had the highest charge of approximately -2.0 eluted last. The elution order was determined by assessing the retention times of several different chromatograms which were produced by injecting the analytes both individually and in various combinations.

We had found that a mobile phase concentration of 0.1 mM citrate buffer was adequate to separate the closest two eluting species. These two species were As(III) and DMAA. Even though the concentration of the buffer might seem very low, the ionic strength of the citrate is stronger than when compared against other buffers. For instance, the strength of the mobile phase ions increase in the sequence:[92]

citrate > SO_4^{-2} > oxalate > I^- > NO_3^- > CrO_4^{-2} > Br^- > SCN^- > formate > acetate > OH^-

In addition, we found that adding 5% (v/v) methanol to the mobile phase was beneficial. This addition of organic solvent allowed for consistency between runs and increased selectivity. The effect of adding organic solvents to the mobile phase in ion-

exchange chromatography was discussed in separate references.[93,94] It is known that organic solvents added to the buffer can sometimes enhance partitioning (column efficiency) on the ion exchange column. That is, it can alter the selectivity and allow greater resolution. Up to 10% organic solvent can be added without degrading column stability. The problems that had arisen without organic solvent (0% (v/v) MeOH) are shown in Figure 16. It was observed that resolution between As(III) and DMAA was difficult to maintain and that equilibration times between subsequent runs, which ranged from 20 - 60 min. in figure, were needlessly long. In Figure 17, we observed that baseline resolution was consistent over several runs when using 5% (v/v) methanol and equilibration times were 7 minutes.

As mentioned previously, we determined that As(V) was the last eluting species. As(V) eluted significantly later than the other species when using a 0.1 mM citrate mobile phase and therefore we decide to use a gradient elution program to reduce the time for eluting As(V). Examples of possible gradient factors that can be changed to alter selectivity during an elution are pH, ionic strength, and flow rate. A gradient of ionic strength and flow rate was chosen because a change in ionic strength usually requires much shorter equilibration times than a change in pH of more than one unit and flow rate changes do not affect equilibration to the same extent as the other two factors.[95] Changes in the pH of the eluent affect both the character of the ion-exchange media and the equilibrium of the ionizing species and thus require a longer time to equilibrate between different pH solutions.

Figure 16. Chromatograms of arsenicals demonstrating poor resolution using 0% MeOH, 0.1 mM citrate pH 9 eluent. Equilibration times: 20-60 minutes. Conc. 50 $\mu g \bullet mL^{-1}$ arsenic. (1) As(III), (2) DMAA, and (3) MMAA.

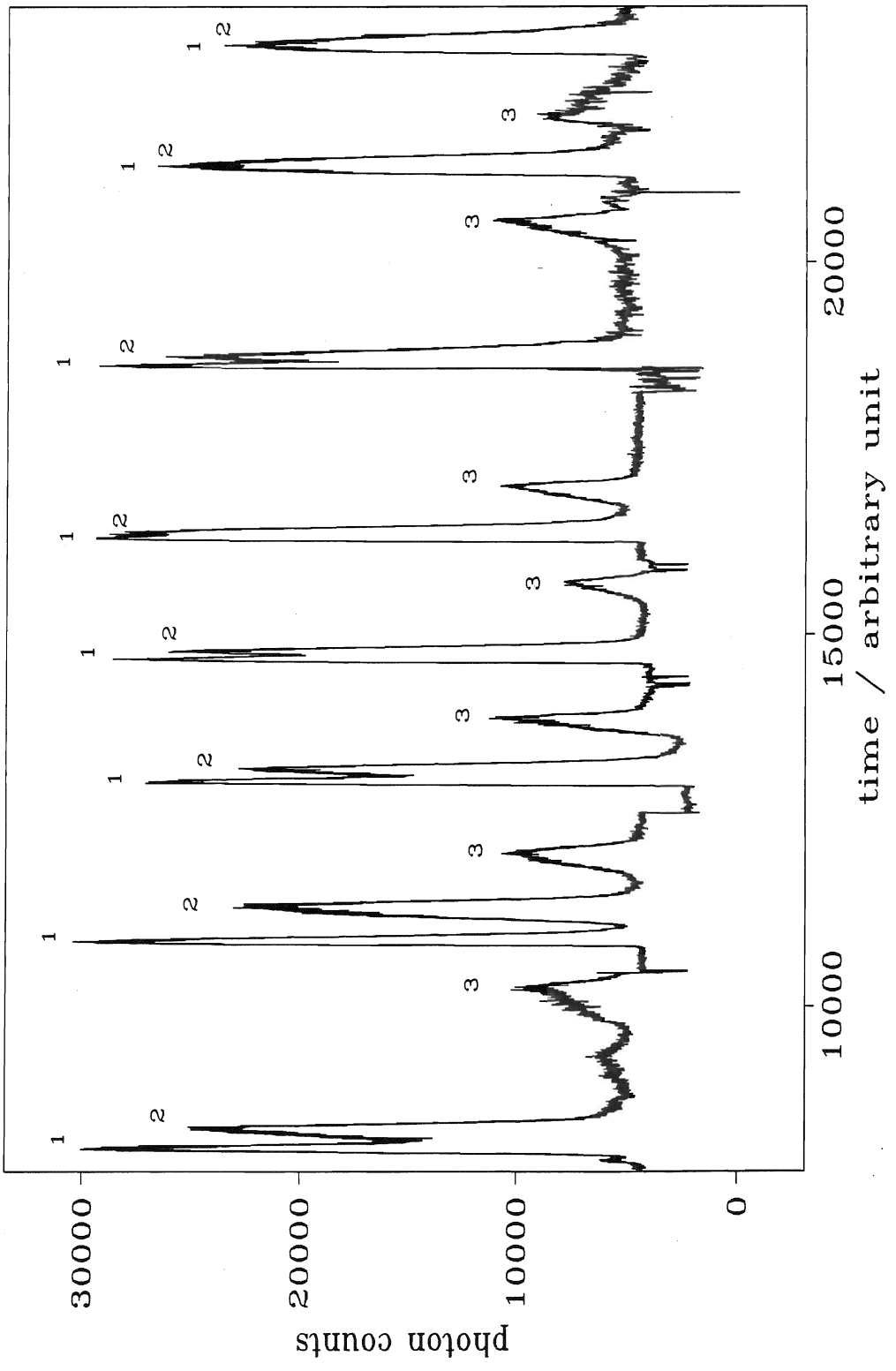
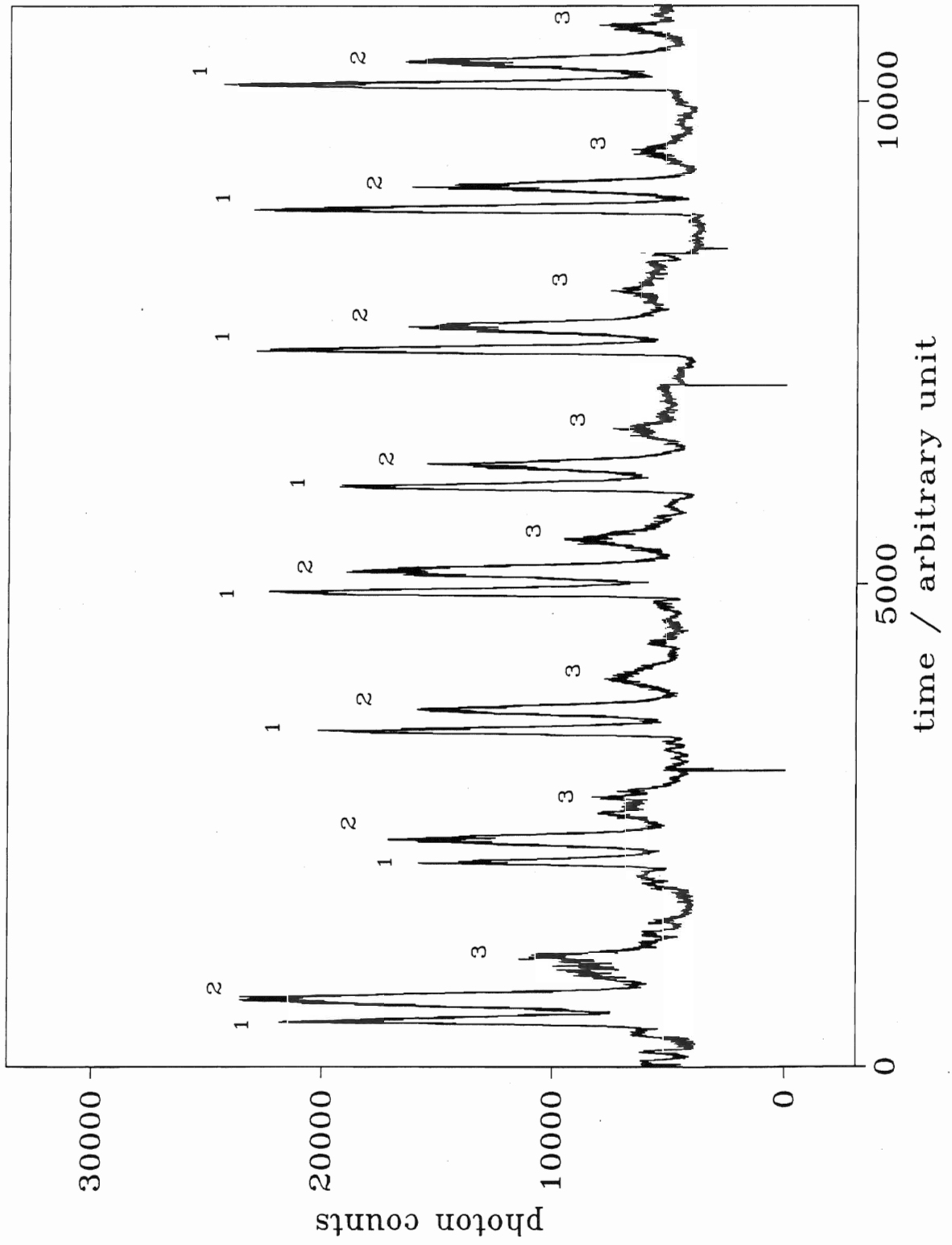


Figure 17. Chromatograms of arsenicals demonstrating consistent resolution using 5% (v/v) MeOH, pH 9 eluent. Equilibration time: 7 minutes. Concentrations: 50 $\mu g \bullet mL^{-1}$ arsenic. (1) As(III); (2) DMAA and (3) MMAA.



A typical chromatogram produced using the gradient elution program described in Table VI is shown in Figure 18. Post-processing of this chromatogram by a simple point averaging program provided with Spectra Calc software was performed to aid in a resolution calculation of adjacent analyte peaks and is shown in Figure 19. Resolution factors between adjacent peaks were calculated according to equation (21). It is appropriate to mention that these factors can be easily calculated from chromatograms which contain Guassian shaped peaks but, in our case, with the continuous flow-through hydride generator producing the signals, the peaks were not ideal, so, we decided that the determination of the resolution factor along with qualitative observations would represent the adequateness of the chromatography. The retention time and peak width measurements are shown directly on Figure 19. The peak widths were measured by extrapolating the relatively straight sides of the peaks to the baseline. Resolution factors along with retention times and peak width values are listed in table Table X. The resolution factor between the two closest eluting species was measured between As(III) and DMAA at a value of 1.1. Even though this value is relatively small, it is obvious after examining the chromatogram that there is significant separation between these two species which meets an objective decided upon at the onset of the chromatography study which was to obtain baseline resolution between all analytes.

Other important information about the chromatography refers to the time required for each analysis and the temperature regulation of the column. The temperature of the column was ambient room temperature. The column kept at room temperature seemed to provide adequate resolution but better resolution and faster analysis times are sometimes possible

Figure 18. Chromatogram of four arsenic species using the gradient method. Concentrations of As(III) and DMAA were 50 $\mu g \bullet mL^{-1}$ and MMAA and As(V) were 100 $\mu g \bullet mL^{-1}$.

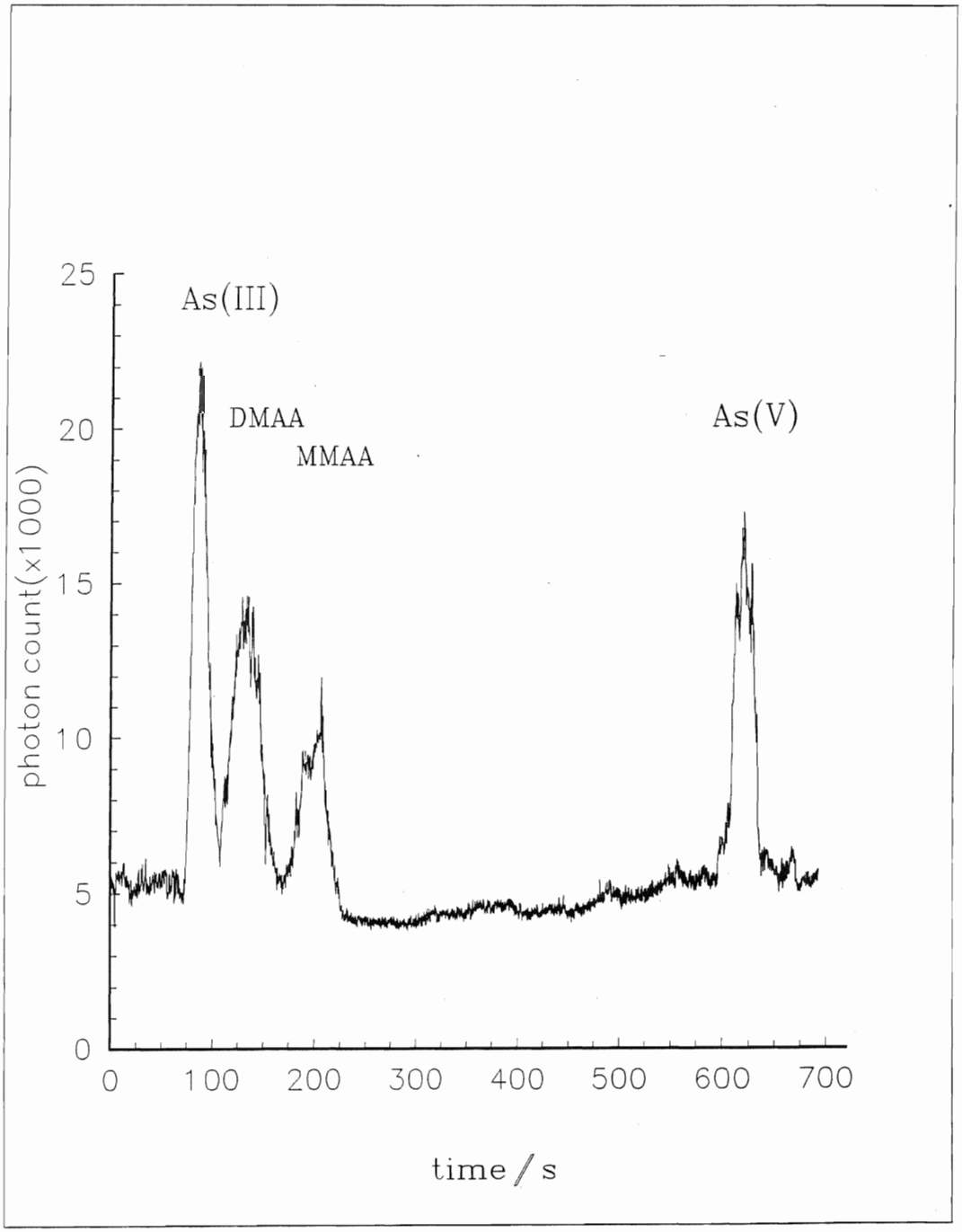


Figure 19. Resolution factor measurements for chromatogram of four arsenic species after using a post-acquire signal averaging program provided by Spectra Calc. This chromatogram was originally acquired as shown in Figure 17.

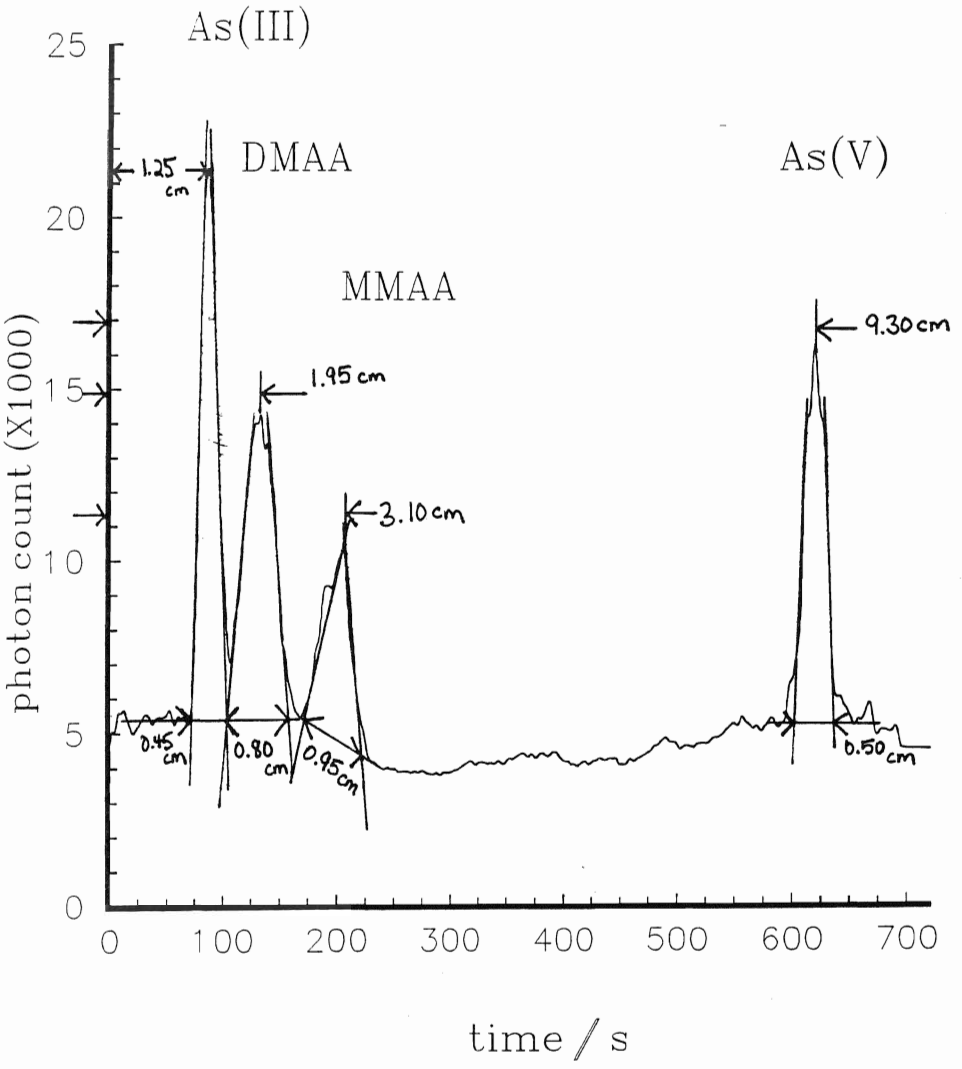


Table X Resolution factors between all species using the values measured from the chromatogram in Figure 19.

Species	Retention time, t_R s	Peak width at base, w cm	Resolution Factor with ensuing species, R
As(III)	$t_{As(III)} = 86$	0.45	R {As(III)-DMAA} = 1.1
DMAA	$t_{DMAA} = 134$	0.80	R {DMAA-MMAA} = 1.3
MMAA	$t_{MMAA} = 212$	0.95	R {MMAA-As(V)} = 8.6
As(V)	$t_{As(V)} = 637$	0.50	N/A

The resolution, R, was calculated using the equation below:

$$R = \frac{2\,(t_{R_2} - t_{R_1})}{w_1 + w_2}$$

with temperatures up to 60 C.[88] The time required for the elution of all four compounds was 12 minutes. The equilibration time between the onset of subsequent runs was 7 minutes. An equilibration time prior to the next run was necessary because the chromatography consisted of a gradient method which required time to flush out the final mobile phase composition and equilibrate to the initial mobile phase composition before the onset of the next run. Therefore a single analysis requires no more than 20 min when including all setup procedures. This time is comparable to other speciation methods reviewed in Table III. Although few methods using a gradient program described the equilibration time necessary between runs there was a general trend observed that reverse-phase mode required shorter equilibration times than IC. For comparison purposes with one of the IC methods that provide equilibrating time information, Spall *et al.*[90] required 20 min equilibration after all analyte eluted. Therefore our analysis time was acceptable. Further comparison of the chromatography relates to a study performed by Shum *et al.*[79] for arsenic speciation of the same four species. The chromatography employed by Shum *et al.* also involved a gradient method but using an IP reverse-phase mode instead of IC. The mobile phase included an organic modifier of methanol to the same proportions as was used in the present study and the order of elution of the four arsenicals was identical. The time for each analysis was 15 min which again compares favourably to the time required here. Therefore based upon the above comparisons the chromatography developed in this laboratory was valid.

Arsine generation using cysteine was found to be pH sensitive, as mentioned previously. Therefore a quick study of the effect of pH on the sensitivity, which was

measured as peak height of MMAA was performed since MMAA was most sensitive to pH changes. From our experiences and the data observed in Table XI, a nitric acid concentration of 0.30 M was used during flow injection hydride generation, though 0.40 M acid provided better MMAA recovery relative to As(III). The higher relative recovery of MMAA with 0.40 M acid was the result of decreased As(III) sensitivity at the higher acid concentration and therefore 0.40 M acid was not used.

During the batchwise procedure, the analytes in the L-cysteine solution required heating to assure complete prereduction. Of the four species, MMAA was the most difficult to reduce since it required the longest reaction times and, as such, will be considered the indicator for the efficiency of heating during the on-line process. Heating of the reagents by the FI method was achieved by placing two pieces of 1 m tubing, i.d. 0.017' and 0.030', through a heated water bath at approximately 90 C. Heat transfer using beakers during the batchwise process was an obvious disadvantage because it was inefficient which resulted from a low surface area to volume ratio for heat transfer. For example, the shortest heating period measuring MMAA recovery for the batch method was 30 s and the time between heating and actual measurement of the analyte by the DCP-AES was approximately 15 min, compared to the FI system in which the analytes were present in the entire 3 m of both heating and cooling tubing for approximately 5-10 s. The recovery of MMAA by the batch and FI systems, relative to As(III), were 52% and 29%, respectively, for the described situations. Therefore we suggest that heat transfer was significantly more efficient in the narrow bore tubing FI system than in the batch system but not complete when considering

Table XI. Effect of acid concentration on the recovery of MMAA during HPLC-FI-HG.

Acid concentration, M	percent recovery MMAA*
0.0	undefined
0.01	0
0.1	4
0.2	14
0.3	20
0.4	24

* standardized against the height of As(III) peak.

that the batch system was capable of 86% recovery of MMAA when the heating period was extended to 3 min. The recoveries of each arsenical during the speciation determination are shown in Table XII. The last 1 m of tubing was used for cooling the solution in order to reduce condensation build-up which would otherwise collect in the transfer tube between the hydride generator and the DCP and, in turn, interrupt smooth carrier gas flow to the plasma torch.

The flow of the argon carrier gas through the hydride generator was also quickly studied. Initially, the flow of argon through the generator was set at 1.6 $l \cdot min^{-1}$, which was the same value as used with the batchwise process. Following a quick study involving the argon flow through the hydride generator, the flow was increased to 2.5 $l \cdot min^{-1}$ because peak height and area values had also increased. The results of the effect of argon flow rate are shown in Table XIII. Another modification made at this time was the stopping of the argon flow through the nebulizer. Argon flow through the nebulizer was stopped because it was not needed and we observed that plasma stability increased significantly at flows over 2.0 $l \cdot min^{-1}$ after the disconnection of the nebulizer. Peak response was not determined for argon flow above 2.5 $l \cdot min^{-1}$ because the plasma became very unstable at these levels.

The final assessment of this method deals with measurement of the analytes with regards to both peak height and peak area. During the many runs performed, we observed that the As(III) was never retained by the column and thus eluted with the solvent front. In turn, the As(III) peak was very narrow and tall for all runs respective to the other species

Table XII. Comparison between peak height and peak area for the arsenic species. Concentrations were: As(III) and DMAA were 50 $\mu g \bullet mL^{-1}$ and MMAA and As(V) were 100 $\mu g \bullet mL^{-1}$.

arsenic species	peak height (photon counts)	peak area (arbitrary counts)
As(III)	1.00	1.00
DMAA	0.50	0.88
MMAA*	0.17	0.29
As(V)*	0.34	0.40

* MMAA and As(V) injection concentrations were 2X the concentration of As(III) and, as such, the values measured from the chromatogram were divided by 2.

Table XIII. Effect of argon flow through the hydride generator on the arsine signal. Analyte was 100 $\mu g \bullet mL^{-1}$ As(III).

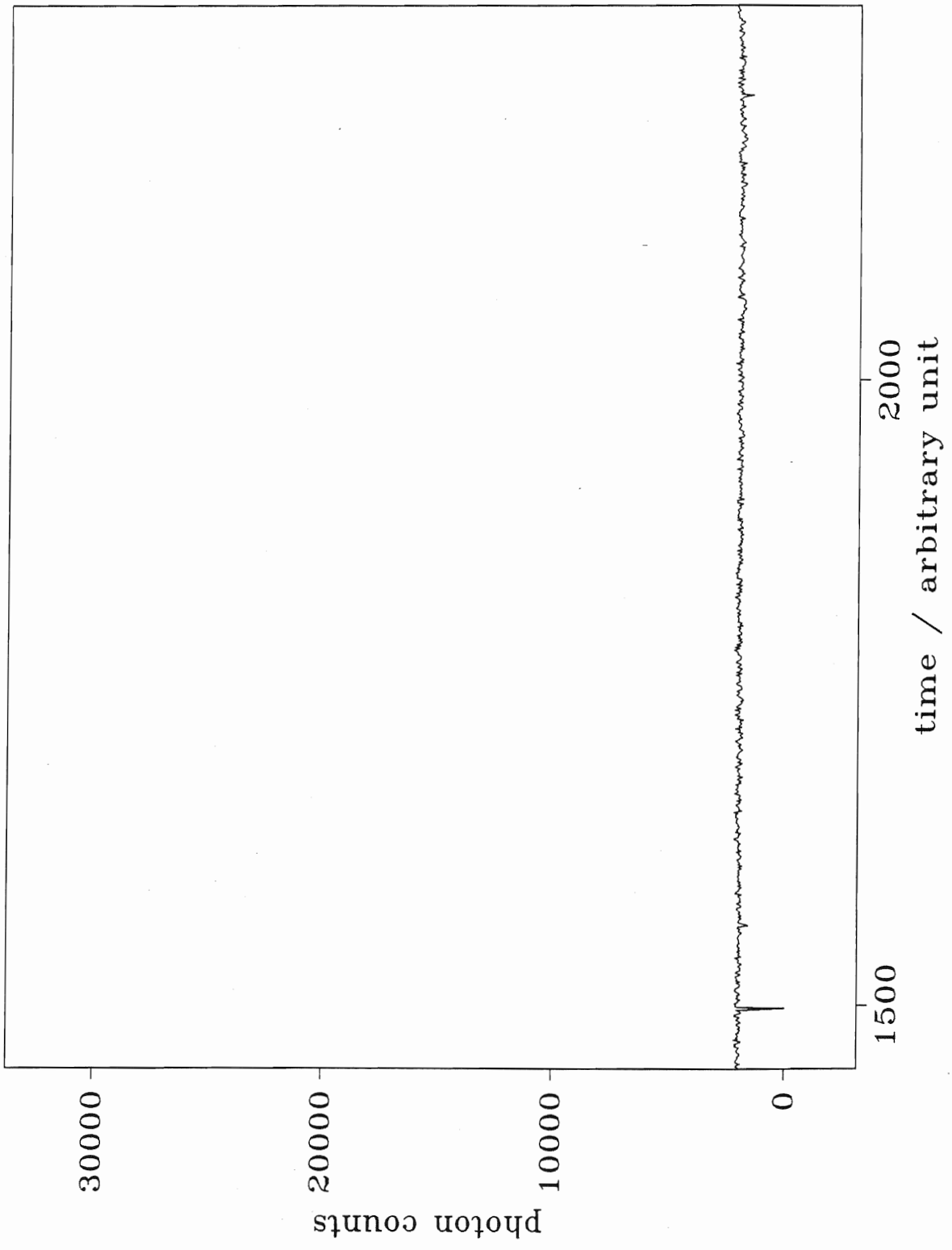
Argon flow	peak height		peak area	
$l\ min^{-1}$	photon counts	relative*	integrated photon counts	relative*
1.6	8693	1.00	332838	1.00
2.5	12979	1.49	412204	1.24

* values are relative to Ar flow of 1.6 $l \bullet min^{-1}$.

and therefore it would be appropriate to measure all other species against As(III) for both peak height and peak area. In Table XII, the results show that recoveries based on peak area are more sensitive and should be used in the future to determine the analyte concentrations.

A blank determination of a sample prepared without the addition of any arsenic species is displayed in Figure 20. No peaks could be detected beyond the noise of the baseline in the 'blank' chromatogram. This is a significant finding because it proves that the peaks produced in the chromatograms with arsenic analyte in the sample were in fact the result of the arsenic species and there is no need to substract the blank from the spiked analyte samples. Other observations from the blank chromatogram were that the noise of the baseline was constant and low and the baseline itself was stable. Therefore the observations from the blank and spiked chromatograms prove that the coupling of HPLC with DCP-AES under the parameters described herein can be applied to arsenic speciation.

Figure 20. Chromatogram of the Blank sample using the operating parameters outlined in Table V and the gradient elution program in Table VI.



CONCLUSIONS

During this project we have demonstrated the ability of L-cysteine to prereduce MMAA and DMAA prior to arsine generation. The conditions required for arsine generation were maximized for all the species. This cysteine reagent was compared against the conventional method using iodide and concentrated hydrochloric acid and was found to rival the sensitivity, being even better for DMAA speciation. The sensitivity obtained for all arsenic species, in the context of this work, by batchwise hydride generation was equivalent.

A continuous arsenic speciation technique was developed. The technique included HPLC to separate the arsenicals by ion-exchange chromatography followed by on-line detection using a continuous hydride generator and determination by DCP-AES. L-cysteine was used to enhance hydride generation in low acid concentration which is less costly and better tolerated by the equipment. The flow injection equipment used for the speciation analysis was assembled from scratch and included a new continuous hydride generator. The further development of the system was limited by time constraints and by the new photon integrator which was built during the final phase of the research. The fine tuning of the system, which is very time consuming, was shortened and thus limited the quality of data which could be acquired. The new FI system, incorporating the new photon integrator, should give detection limits in the range of 100 $ng \bullet mL^{-1}$.

Finally, we have shown that L-cysteine can be used successfully for hydride generation during an arsenic speciation determination. Therefore work can now be directed towards improving sensitivity of the continuous method with the goal of applying this technique to an automated speciation method with high throughput and detection limits below natural arsenic levels.

SUGGESTIONS FOR CONTINUED WORK

The groundwork has been done into the preparation and installation of the FI system. Therefore work should now be performed into maximizing the arsenic signal. It would be beneficial to study the effects of the FI operating parameters. For instance, more data about the effect of acid concentration, the effect of borohydride concentration, and the effect of L-cysteine concentration on arsine generation must be gathered. Increasing heating efficiency should also be pursued by either using a hotter bath, such as 150 C paraffin bath, or by slowing reagent flow and increasing reaction time. The foaming problem and its adverse effects on signal shape could be eliminated with antifoaming agents such as tributyl phosphate. The proportion of methanol in the mobile phase might be decreased while maintaining resolution and in turn decrease foaming. Finally, the effect of buffer type on both the analyte selectivity during elution and the arsine generation should be studied.

APPENDIX 1.

Electronic Modifications of the DCP-AES Photomultiplier by interfacing to A/D for Photon Signal Integrating

During the final period of this project a photon counting device was developed because of electronic equipment failure in the commercial instrument. Different configurations were tested before finding an acceptable design. The initial design was based on solely transistor-transistor logic (TTL) but it was soon discovered that the response time of this system was slower than the photon event from the photomultiplier tube (PMT). Emitter-coupled logic (ECL) was the essential replacement over TTL because ECL based logic operates at higher speeds which is imperative in order to measure the photon events. TTL, though it is still one of the most popular logic systems in use, operates at speeds of 25 MHz, whereas ECL can operate at 100-500 MHz, which is the highest speed possible for bipolar logic. The events from the PMT were found to be on the order of nanoseconds after measurement with an oscilloscope. A photon event produced by the PMT is basically the minimum required number of photons striking the PMT that produce a measurable response from the PMT.

The photon counting device incorporated true ECL and ECL hybrid hardware. All chips used were all Motorola's emitter-coupled logic (MECL) units. ECL is defined as a non-saturating form of digital logic which eliminates transistor storage time, thereby

permitting very high speed operation. ECL is characterized by high impedance inputs, high voltage gain, and low output impedance for good line driving. Other advantages include constant supply current, very low noise generation, which will be discussed later.[1]

The electronic design of the photon integrator used here was based on the circuit developed by Darland *et al.*[2] Modifications to this circuit were made with regards to the hardware available at the present time. As such modifications were mainly the different selection of ECL chips. The entire photon integrator is comprised of (a) a video amplifier, (b) an ECL comparator, (c) an ECL translator, and (d) an ECL monostable. Even though all the chips were ECL based, each chip was unique and performed a separate task. which were:

(a) The video amp was an integrated circuit (IC) differential video amplifier which is an ECL hybrid chip. The video amp is a high speed linear device and is used because it amplifies the original input signal from the PMT and produces complementary and stretched output signals. Complementary output signals are defined as the generation of two signals which are equal in magnitude but are opposite phase. Advantages of complementary output signals from the video amp include:

(i) Complementary signals are balanced because the sum of the amplitudes of the two signals is constant. For instance, if the one of the outputs becomes more negative then the other output becomes more positive

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1. Blood, W.R., In *MECL System Design Handbook*, 4th ed., Motorola Inc., 1983, pp. vi-x.

2 Darland, E.J., Hornshuh, J.E., Enke, C.G., and Leroi, G.E., Anal. Chem., 1979, 51, 245-250.

but the sum of the two signals is always constant, and vice-versa. Therefore, current demand is stable and constant.

(ii) Neutral impact on the power supply since the current demand of the IC is constant.

(iii) The sensitivity of the video amp has doubled when considering that the difference between the two complementary output signals, which are opposite in phase, has doubled, as compared to the amplitude of a single output signal.

(iv) Complementary signal are imperative for interfacing to the linear ECL comparator.

(v) High-noise immunity because of output balancing.

The gain of the video amp was internally fixed at 100. Therefore input signals from the PMT, which are on the order of 1 mV, are amplified to 100 mV. Also, the video amp is pulse width independent and produces output signals that are longer duration than the input signal. The output signals are approximately 5-15 ns wide.. Output signals are approximately 10 ns wide. The operating range for this amp is 5-500 000 pulses per second resulting in a dynamic range of 10^6.

(b) The comparator is used as an interface between an analog input signal from the video amp and driving the inputs of the ECL translator. It is essentially a linear, high-speed, high-gain amplifier. Its most important application was threshold adjustment. The input signal to the comparator is adjustable and is used to set a threshold level that discriminates between background noise and signals from the PMT. The output from the comparator is also complementary and, as such, has the advantages listed above with the video amp. Pulses from the comparator were 15-20 ns wide.

(c) The ECL translator is an analog hybrid chip. This device interfaces an ECL analog hybrid to standard ECL logic by translating a linear input signal to a digital output signal.

(d) The ECL monostable was used to interface the ECL based photon integrator with an external TTL based analog to digital (A/D) convertor. The device slows down the very fast ECL input signal to produce longer output pulses which are compatible with slower TTL and TTL compatible components, such as the diode and signal integrator shown in the diagram. An ECL monostable is characterised by the capacitative coupling of the output of one gate to the input of another gate. As a result, the monostable circuit sits in one state until it is momentarily forced to the other state by an input pulse. It will return to the original state after a time delay determined by the capacitor value and the input current.[3] The stretched output signals were used to directly power the diode and integrating circuit.

The output from the photon integrator was directed to an Analog/Digital convertor built by the Electronics shop before it was sent to the IBM Compatible computer.

The photon integrator was placed within the PMT housing in order to minimize the length of the connecting cable between the PMT and the photon integrator and thus decrease any line interferences where it would be most sensitive. The cables from the PMT to the photon integrator and from the photon integrator to the A/D convertor were coaxial with

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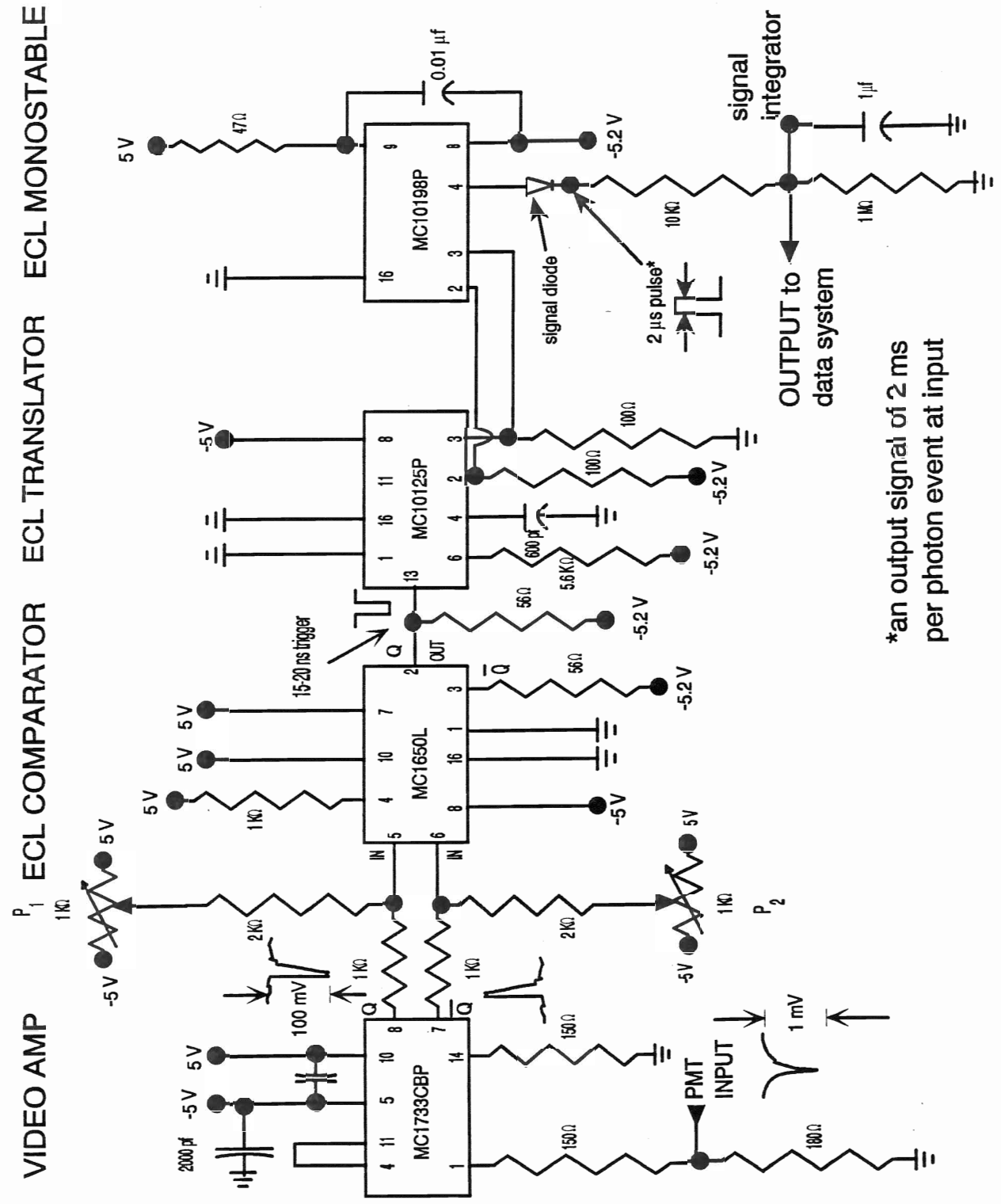
3 Horowitz, P., and Hill, W. pp. 517-518.

BNC connectors. The dimensions of the photon integrator box was 15 × 8 × 8 cm (l × w × h). The two variable resistors were 10-turn 1 KΩ pots. The adjusting knobs for the pots were attached at the PMT housing and were easily accessible. The pots were necessary because the day-to-day stability of the photon integrator was slightly inconsistent and therefore the sensitivity of the photon integrator to both the background noise and PMT signals needed regular adjustment.

The photon integrator is very sensitive to external interferences. For example, the stability of the photon integrator was adversely affected by the temperature. The authors, who originally developed the integrator, reported that a change in temperature from 30 to 50 C was accompanied by a 30% decrease in sensitivity.[2] They used a temperature controller to maintain a consistent environment. Similar effects were observed here. The sensitivity would decrease as the integrator warmed-up. The warming of the integrator was accentuated because of the fact it was placed in the closed housing. Therefore an exhaust cooling fan was placed inside the box and a cooling fan was directed over the PMT housing. Another source of external interference was the d.c. arc, which was approximately 25 cm from the counting device. The d.c. arc was a significant source of high-frequency interference which adversely affected the sensitivity of the photon integrator. The threshold levels of the integrator were adjusted in order to compensate for the arc noise which, unfortunately, was accompanied with a loss of sensitivity. The photon integrator had minimal shielding, only.

In the future, it would be beneficial to place a cooling water coil through the housing next to the integrator and increase the shielding of the unit either using an internal Faraday cage or triaxial shielding.

Figure 21. Schematics for the photon integrator.



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